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PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES INDIA

1957

PART IV]

SECTION A

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NITROGEN TRANSFORMATIONS IN SOIL WITH STRAW

BY A. K. BHATTACHARYA AND P. N. AWASTHI

(Department of Chemistry, Saugar University)

Read at the 26th Annual Session of the Academy held at the Aligarh Muslim University
on 4th February 1957

It is well known that of all the soil nutrients Nitrogen is indisputably by far the most important. Nitrogen, as is well known, is the most important nutrient of the plant kingdom also and is an essential constituent of the fundamental living matter, the protoplasm. Indeed the well-known nitrogen cycle consisting of its various transformations from atmosphere to soil, soil to plant, plant to animal, animal to soil and air again, is usually termed "the wheel of life" which indicates its importance in nature and to the living world. And nitrogen is the most commonly deficient nutrient in the arable soils of the world.

The majority of Indian soils are poor in nitrogen. Dr. J. A. Voelekar¹ in his *Improvement of Indian Agriculture* reports, "on looking into the analysis of Indian soils, which have been recorded and others which I have made myself, I find that with the possible exception of the black cotton soils, Indian soils are generally deficient, both in organic matter and nitrogen." The Royal Commission on Agriculture in India² (1928) also states "of the principal plant food materials in which the soil of India are deficient, by far the most important is nitrogen and the manurial problem in India is in the main one of nitrogen deficiency."

Experiments with Indian soils point out yet another very important factor for the poverty of Indian soils. This is its deficiency in humus content. The Agricultural Chemist to the Government of U.P. (India), in one of his recent publications, has stated the following:—

“In brief utilising as much organic matter as is available and supplementing this with inorganic fertilizers holds the best promise for solving our food problem.”

B. Vishwanath³ agrees with the above view when he says that “Analysis of soils supplemented by field observations in different parts of the country established general deficiency in organic matter, nitrogen and phosphorus”.

The nitrogen deficiency of Indian soils is greatly due to the climatic conditions prevailing in the sub-continent. To meet the deficiency of nitrogen and to increase productivity, the addition of artificial fertilizers in the form of ammonium salts to the soil is generally adopted in all countries. These salts undoubtedly increase the crop production but the results of experiments at Rothamsted⁴ and other places⁵ show that a good deal of salts thus added is lost to the soil without being of use to the plants. Indeed the loss of nitrogen from artificial manures is often even more than 50% as is indicated by the following statement of Russell.⁶

“Crops that respond to nitrogen manuring commonly take up and fix in their mature tissue between one-third and one-half of the nitrogen added as sulphate of ammonia, and a rather higher proportion of nitrogen added as nitrate, the remainder is lost to the crop and usually to the soil—though its fate has not been too well determined.” Similar loss has also been reported by G. V. Jacks,⁷ Bracken and Greaves.⁸ All these results point to the conclusion that nitrogen is being continuously lost from cultivated soil, in all kinds of practices, soils to which large amount of synthetic fertilizers are added and also even from soils which are only broken and tilled for cultivation. This loss of nitrogen has been explained by various theories, *e.g.*, Leaching, Bacterial, Chemical, Photochemical, etc., but all the theories come to agree on, one and the same point that the soil loses its nitrogen in some form or the other in due course of time. The authors, therefore, without discussing the merits and demerits of the theories have attempted to conserve nitrogen with material such as straw, which although very cheap are often overlooked as a conservator.

We have, therefore, attempted to show that wheat straw which is often uncared can play an important role in promoting the nitrogen status of the soil. This substance was chosen due to the cheapness of its cost and easy availability to the Indian farmers in general. Observations were recorded

for the loss of nitrogen, oxidation of carbon and other physical and chemical properties.

Three grams of wheat straw in a finely powdered form per pound of the soil was taken with 50 ml. of water and was mixed with soil containing 3 grams per pound of ammonium nitrate. Another set containing 3 grams of straw per pound of soil was taken. These samples were taken in duplicate in glass jars and these were exposed daily for eight hours in sunlight.

ANALYSIS OF WHEAT STRAW

Total Nitrogen	0.5600%
Total Carbon	39.9010%
Ash	8.6800%
Loss on ignition	92.0000%
Moisture	4.3260%
Magnesium oxide	0.9090%
Silicon dioxide	7.2408%
Calcium oxide	0.2300%
Potassium oxide	0.6968%
Phosphorus pentoxide	0.0658%

TABLE I

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Ammonium Nitrate

Period of Exposure	Total Carbon %	Total Nitrogen %	Total $\text{NH}_3\text{—N}$ %	Total $\text{NO}_3\text{—N}$ %	% Loss
Original soil	0.4043	0.0348	0.0004	0.0010	..
0 Day ..	0.4043	0.2658	0.1531	0.1545	..
1 Month ..	0.3885	0.1595	0.0763	0.0898	39.99
2 Months ..	0.3736	0.1431	0.0691	0.0788	46.16
3 Months ..	0.3591	0.1266	0.0587	0.0678	52.38
4 Months ..	0.3441	0.1152	0.0520	0.0602	56.66
5 Months ..	0.3295	0.1037	0.0452	0.0527	60.99
6 Months ..	0.3137	0.0968	0.0413	0.0479	63.58
7 Months ..	0.2980	0.0899	0.0373	0.0431	66.17
8 Months ..	0.2838	0.0792	0.0338	0.0383	70.20

TABLE II

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Wheat Straw + 3 gm. of Ammonium Nitrate

Period of Exposure %	Total Carbon %	Total Carbon Oxidised %	Total Nitrogen %	Decrease in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010	..
0 Day ..	0.6665	..	0.2695	..	0.1535	0.1555	..
1 Month ..	0.6132	0.0533	0.1928	0.0766	0.0998	0.0762	28.46
2 Months..	0.5732	0.0933	0.1837	0.0857	0.0867	0.0684	31.83
3 Months..	0.5399	0.1266	0.1740	0.0954	0.0737	0.0606	35.40
4 Months..	0.5133	0.1532	0.1736	0.0958	0.0660	0.0598	35.71
5 Months..	0.4866	0.1799	0.1730	0.0964	0.0583	0.0590	35.80
6 Months..	0.4533	0.2132	0.1727	0.0967	0.0552	0.0575	35.91
7 Months..	0.4266	0.2399	0.1659	0.1036	0.0522	0.0559	38.44
8 Months..	0.3999	0.2666	0.1571	0.1123	0.0514	0.0544	41.69

TABLE III

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Straw

Period of Exposure	Total Carbon %	Carbon oxidised %	Total Nitrogen %	Increase in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010
0 Day ..	0.6665	..	0.0385	..	0.0004	0.0010
1 Month ..	0.6325	0.0340	0.0395	0.0010	0.0004	0.0010
2 Months ..	0.5998	0.0667	0.0405	0.0020	0.0004	0.0010
3 Months ..	0.5652	0.1013	0.0415	0.0030	0.0004	0.0010
4 Months ..	0.5405	0.1260	0.0428	0.0043	0.0004	0.0010
5 Months ..	0.5158	0.1507	0.0443	0.0058	0.0004	0.0010
6 Months ..	0.4905	0.1760	0.0456	0.0071	0.0005	0.0010
7 Months ..	0.4678	0.1987	0.0463	0.0078	0.0005	0.0010
8 Months ..	0.4505	0.2160	0.0463	0.0079	0.0006	0.0010

DISCUSSION

Due to recent introduction of tractors all over the world the farms are being denuded of the cattle population and hence there is a scarcity of farm-yard manure, which was practically the most important manure in crop production all over the world. In Europe to-day many farms are being run without animals altogether. Hence the cereal producing farms have a surplus of straw and, therefore, utilisation of this material appears to be a burning problem of modern agriculture.

A continuous but comparatively slow loss of nitrogen was observed (Table I). A loss of 41·69% of nitrogen was observed in 8 months time in presence of wheat straw whilst a loss of 70·20% was noted when ammonium nitrate alone was mixed with the soil. Both the total ammoniacal and nitric nitrogen showed a steady decrease. The oxidation of carbon was vigorous.

The ammoniacal and nitric nitrogen increased considerably at the end (Table III) whilst this was not so much marked in the beginning. The rate of increase in total nitrogen was faster in the beginning but it falls off at the end. A total increase of 2·6% to 21·8% of nitrogen was observed from 1st to 8th month exposure whilst the oxidation of carbon was from 5·1% to 32·4% in the same time. In three months time the oxidation of carbon was 15·2%, in six months it rose to 26·4% while the total increase of nitrogen during these periods was 7·8% and 18·7%.

The following general results were met with wheat straw:—

- (1) Organic carbon content of the system decreases with the time.
- (2) There is a concomitant increase of nitrogen in every case.
- (3) The efficiency decreases with the progress of time.
- (4) The rate of increase of nitrogen is faster in the beginning but it falls off as the period of exposure increases.

It is therefore quite clear from the above results that the loss of nitrogen decreases in presence of straw than in its absence. It therefore proves beyond doubt that the addition of such a cheap product such as wheat straw can act as an efficient conservator if used along with ammonical fertilizers.

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NITROGEN TRANSFORMATION IN SOIL WITH SAWDUST

BY A. K. BHATTACHARYA AND P. N. AWASTHI

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WE have shown¹ that the amount of nitrogen added as inorganic fertilizer is lost to a major extent to the atmosphere without being of much use to the plants. This has been explained due to the formation and decomposition of ammonium nitrite, which is greatly accelerated by light temperature, etc. These and other processes are going on in the soil, so that the soil is always losing small or moderately large amounts of nitrogen according to the nature and extent of its nitrogen content and the conditions to which it is exposed. Soils must therefore, possess some methods for gaining it, in an attempt to balance these losses. It has always been observed that arable soils if allowed to revert either to natural forest or to a pasture, gradually gain nitrogen and organic matter. In Broadbalk field in Rothamsted Experimental Station, for example, a plot was allowed to revert to natural prairie in 1882 by cutting out all trees and bushes at regular intervals. After 22 years, in 1904, on analysing the soil, A.D. Hall² found the following results:—

	Nitrogen per cent. in	
	1886	1904
1st 9 inches of soil	.. 0.108	0.145
2nd 9 inches of soil	.. 0.070	0.095
3rd 9 inches of soil	.. 0.058	0.084

The capacity of the soil to regain nitrogen has been the subject of much investigation. Dhar and Mukerjee³ have found that sterilized soils fix much nitrogen when exposed to light. Pant, Kapoor and Chatterjee, etc., have found that energy-rich materials like cellulose, green leaves, etc., fix nitrogen in soil. The decrease in losses of nitrogen in urea, hippuric acid, gelatin and ammonium salts have been observed by Dhar's school, when carbonaceous materials are added to the soil. Subrahmanyam⁴ has observed that addition of cellulosic materials is fairly effective in checking loss of nitrogen following addition of ammonium fertilizers to soils, specially under Indian climatic conditions. Tillers of fine textured soil in U.S.A. have found that incorporation of sawdust with chemical fertilizer has greatly improved the physical properties of the soil. Apple pomace is often found in the vicinity of sawdust piles.

Experiments were therefore conducted to see whether it can be used for the enrichment of the soil and thus increase in food production by a cheap method.

ANALYSIS OF SAWDUST

Total Nitrogen	0.8428%
Total Carbon	45.2800%
Ash	10.8600%
Silica	4.3600%
Sesquioxide	1.5899%
Calcium oxide	1.1706%
Magnesium oxide	0.6720%
Potassium oxide	1.2785%
Phosphorous	0.3871%

A. Brutani in his book on *Uses of Waste Materials*, 1923 edition, page 185, has given the following representative analysis of a typical sawdust of deciduous trees:—

Water	9.8%
Crude protein	0.5%
Crude fat	0.8%
N-Free extract	37.3%
Crude fibre	49.9%
Mineral substances	1.7%

TABLE I

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Ammonium Nitrate

Period of Exposure	Total Carbon %	Total Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	0.0348	0.0004	0.0010	..
0 Day ..	0.4043	0.2658	0.1531	0.1545	..
1 Month ..	0.3885	0.1595	0.0763	0.0898	39.99
2 Months ..	0.3736	0.1431	0.0691	0.0788	46.16
3 Months ..	0.3591	0.1266	0.0587	0.0678	52.38
4 Months ..	0.3441	0.1152	0.0520	0.0602	56.66
5 Months ..	0.3295	0.1037	0.0452	0.0527	60.99
6 Months ..	0.3137	0.0968	0.0413	0.0479	63.58
7 Months ..	0.2980	0.0899	0.0373	0.0431	66.17
8 Months ..	0.2838	0.0792	0.0338	0.0383	70.20

TABLE II

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Sawdust + 3 gm. of Ammonium Nitrate

Period of Exposure	Total Carbon %	Carbon Oxidised %	Total Nitrogen %	Decrease in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010	..
0 Day ..	0.7018	..	0.2713	..	0.1531	0.1545	..
1 Month ..	0.6908	0.0210	0.1844	0.0868	0.0964	0.0726	32.01
2 Months..	0.6597	0.0421	0.1702	0.1010	0.0827	0.0648	37.25
3 Months..	0.6387	0.0631	0.1596	0.1116	0.0689	0.0571	41.14
4 Months..	0.6106	0.0912	0.1513	0.1200	0.0612	0.0556	44.23
5 Months..	0.5966	0.1052	0.1425	0.1278	0.0536	0.0541	47.47
6 Months..	0.5755	0.1263	0.1386	0.1326	0.0505	0.0525	48.90
7 Months..	0.5545	0.1473	0.1299	0.1413	0.0474	0.0510	52.10
8 Months..	0.5404	0.1614	0.1232	0.1480	0.0459	0.0500	54.56

TABLE III

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Sawdust

Period of Exposure	Total Carbon %	Carbon Oxidised %	Total Nitrogen %	Increase in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010
0 Day ..	0.7018	..	0.0403	..	0.0004	0.0010
1 Month ..	0.6899	0.0119	0.0410	0.0007	0.0004	0.0010
2 Months ..	0.6793	0.0225	0.0418	0.0015	0.0004	0.0010
3 Months ..	0.6568	0.0450	0.0425	0.0022	0.0004	0.0010
4 Months ..	0.6308	0.0710	0.0427	0.0024	0.0004	0.0010
5 Months ..	0.6168	0.0850	0.0430	0.0027	0.0005	0.0011
6 Months ..	0.6007	0.1011	0.0432	0.0029	0.0005	0.0012
7 Months ..	0.5860	0.1158	0.0434	0.0031	0.0005	0.0012
8 Months ..	0.5768	0.1250	0.0436	0.0033	0.0005	0.0012

DISCUSSION

The percentage loss of nitrogen was continuous (Table II). A constant loss of ammoniacal and nitric nitrogen was also seen. The total loss of nitrogen was found to be 54.56% in eight months time with sawdust as compared to 70.20% (Table I) when ammonium nitrate was used with the soil alone. The conservation of nitrogen was slow and the loss of nitrogen was fast because of the oxidation of sawdust when exposed to light with the soil is slow.

The effect produced by sawdust alone when mixed with the soil and exposed to sunlight shows (Table III) that the ammoniacal nitrogen was practically unchanged up to 4 months exposure and then it slowly increased. A slow increase in the nitric nitrogen was also observed at the end. The total nitrogen of the system also increased slowly at first and increased towards the end sufficiently. The oxidation of carbon was 1.7% in the first month, 3.2%, 6.4%, 10.7%, 12.1%, 14.4%, 16.5% and 17.8% in the second, third, fourth, fifth, sixth, seventh and eighth months respectively. A net increase of total nitrogen was found to be 1.8% to 8.3% in eight months time.

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INFLUENCE OF CHEMICAL CONSTITUTION ON THE ROTATORY POWER OF OPTICALLY ACTIVE COMPOUNDS

Part III. Rotatory Dispersion of Salts of Benzoic-*o*-, *m*-, and *p*-Hydroxy Benzoic and *o*- and *p*-Amino Benzoic Acids with Brucine

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Read at the 26th Annual Session of the Academy held at the Aligarh Muslim University
on 4th February 1957

ALKALOIDS which are optically active nitrogenous bases, have been used for the resolution of optically active compounds containing an acidic group since the days of Pasteur.¹ However, till the beginning of this century, these compounds do not appear to have been used for the purpose of study of optical activity in relation to chemical constitution. Minguin² seems to have initiated the use of strychnine by investigating the optical properties of its salts with many organic acids in a mixture of benzyl and ethyl alcohols. Hilditch³ then used alkaloids like strychnine, brucine, quinine, etc., for preparing optically active salts and employed them for a study of this problem. Since then a great deal of work has been done by subsequent workers by using salts of alkaloids with aliphatic and aromatic acids.

In the present communication the authors have prepared benzoic-, *o*-, *m*- and *p*-hydroxy benzoic-, *o*- and *p*-amino benzoic acid salts of brucine and studied their rotatory dispersion in various solvents.

The effect of replacing the hydrogen atom by electropositive or electronegative groups on the rotatory power of optically active salts of alkaloids does not appear to have received much attention and little is known about the polar effect of the groups in these types of compounds. This aspect has been explored by comparing the optical rotatory power of the compounds in question.

EXPERIMENTAL

These salts were prepared by mixing brucine and the acid in equimolecular proportions in water or ethyl alcohol. The solutions thus obtained were filtered and concentrated when the crystals of the salts separated out either on standing or on scratching with a glass rod. They were then repeatedly recrystallised out of water, ethyl alcohol, ethyl acetate, acetone or a mixture of ethyl alcohol or ethyl acetate.

Brucine benzoate.⁴—It is a white crystalline powder soluble in water, methyl alcohol, ethyl alcohol, pyridine, glacial acetic acid, acetone dioxan and chloroform and practically insoluble in ether, benzene and carbon tetrachloride, m.p. 94.5°C . Found: C = 64.20%, H = 6.50% and N = 5.11%. $\text{C}_{30}\text{H}_{32}\text{O}_6\text{N}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ requires C = 64.17%, H = 6.59% and N = 4.99%.

Brucine-o-hydroxy-benzoate.⁴—It crystallises as white needle-shaped crystals. It is soluble in pyridine, chloroform and glacial acetic acid, sparingly so in ethyl alcohol, methyl alcohol, acetone and water and practically insoluble in ether, carbon tetrachloride and benzene. m.p. $250\text{--}54^{\circ}\text{d}$. Found: C = 67.80%, H = 6.1% and N = 5.6%. $\text{C}_{30}\text{H}_{32}\text{O}_7\text{N}$ requires C = 67.66%, H = 6.00% and N = 5.26%.

Brucine-m-hydroxy benzoate.—This compound crystallises as silky white needles. It is freely soluble in pyridine and glacial acetic acid, less so in methyl alcohol, ethyl alcohol and dioxan, very sparingly soluble in water, ethyl acetate, acetone and chloroform and practically insoluble in ether, benzene and carbon tetrachloride, m.p. 162°C . Found: C = 68.00%, H = 5.72% and N = 5.40%. $\text{C}_{30}\text{H}_{32}\text{O}_7\text{N}_2$ requires C = 67.66%, H = 6.00% and N = 5.26%.

Brucine p-hydroxy benzoate.—It is a white crystalline solid soluble in pyridine, glacial acetic acid, methyl alcohol, ethyl alcohol and dioxan, sparingly so in acetone, ethyl acetate and water and practically insoluble in ether, benzene, carbon tetrachloride and chloroform. m.p. 150°C , Found: C = 68.10%, H = 6.32% and N = 5.27%. $\text{C}_{30}\text{H}_{32}\text{O}_7\text{N}_2$ requires C = 67.66%, H = 6.00% and N = 5.26%.

Brucine-o-aminobenzoate.⁴—This compound crystallises as cream coloured crystals which are fairly soluble in pyridine, chloroform and glacial acetic acid, less so in methyl alcohol, ethyl alcohol and dioxan, very sparingly soluble in acetone and ethyl acetate and practically insoluble in benzene, ether and carbon tetrachloride, m.p. $155\text{--}57^{\circ}\text{C}$. Found: C = 67.90%, H = 6.39% and N = 7.66%. $\text{C}_{30}\text{H}_{33}\text{O}_6\text{N}_3$ requires C = 67.79%, H = 6.21% and N = 7.9%.

The rotatory power determinations were made in a 2.2 d.cm. polarimeter tube at room temperature (about 30°C .). The values of λ_0 calculated from the dispersion formula are given in Tables II to VII and are expressed as μ or 10^{-4} cm .

DISCUSSION

Nature of the rotatory dispersion.—The rotatory dispersion of these brucine salts has been determined in the visible region of the spectrum for eleven wavelengths (6708 Å.U. to 4358 Å.U.) in various solvents and the results are recorded in Tables II to VII. The dispersion is found to be "simple" as it follows Drudes' one-term equation, $(\alpha) = \frac{K_0}{\lambda^2 - \lambda_0^2}$. In this equation K_0 and λ_0 are constants representing the absolute rotation of the substance under examination and the wavelength of the dominant absorption band of its molecule in the ultraviolet region of the spectrum respectively.

TABLE I

No.	Brucine Salts of	[α] $^{30^\circ}_{5461}$			
		Chloro- form (5.2)*	Pyri- dine (12.4)	Ethyl- alcohol (25.8)	Methyl- alcohol (31.2)
1	Benzoic Acid ..	—32.48° (6.898)†	—82.70° (17.08)	—35.45° (7.341)	—40.00° (8.039)
2	<i>o</i> -amino-benzoic acid ..	—16.59 (2.589)	103.60 (23.79)	40.00 (9.456)	52.70 (12.77)
3	<i>o</i> -hydroxy-benzoic acid +	19.50 (3.938)	47.27 (9.670)	..	—
4	<i>m</i> -hydroxy-benzoic acid	79.09 (16.07)	30.90 (6.500)	36.36 (7.240)
5	<i>p</i> -amino-benzoic acid —	30.69 (6.427)	95.30 (20.46)	40.90 (9.665)	47.17 (10.36)
6	<i>p</i> -hydroxy-benzoic acid	73.60 (14.65)	30.00 (6.233)	40.90 (8.736)

* Represents the dielectric constants of solvents.

† Represents the values of K_0 , rotation constant.

The observed values of the optical rotatory power (O) for different wavelengths closely agree with the corresponding calculated values (C). In Table II to Table VII given the difference *o-c* with few exceptions these differences are within the experimental error.

TABLE IV
Brucine p-aminobenzoate

Solvent	Chloroform	Ethyl alcohol	Methyl alcohol	Pyridine		
Concentration in gm./100 c.c.	2.00	0.500	0.500	0.500		
Calculated $\left\{ \begin{array}{l} (\alpha) \\ (\lambda_0) \end{array} \right.$	$\frac{6.427}{\lambda^2 - 0.086}$ 0.2932	$\frac{9.665}{\lambda^2 - 0.0619}$ 0.2488	$\frac{10.36}{\lambda^2 - 0.0782}$ 0.2796	$\frac{20.46}{\lambda^2 - 0.0823}$ 0.2869		
Line	Obs. (α) (O)	Cal. (α) (C)	(O-C)	Obs. (α) (O)	Cal. (α) (C)	(O-C)
Li 6708	-17.50°	-17.66°	-0.16°	-25.45°	-24.91°	+0.54°
Cd 6438	19.31	19.56	-0.25	27.27	27.42	-0.15
Li 6108	22.95	22.43	+0.52	31.80	31.12	+0.68
Na 5803	24.90	24.61	+0.29	34.54	33.88	+0.66
Hg 5780	25.90	25.90	0.00	36.36	35.50	+0.86
Hg 5461	30.69	30.29	+0.40	40.90	40.90	0.00
Cd 5085	37.27	37.22	+0.05	49.09	49.10	-0.01
Cd 4799	44.50	44.50	0.00	56.36	57.36	-1.00
Cd 4678	47.70	48.37	-0.67	61.80	61.56	+0.24
Li 4602	50.68	51.09	-0.41	64.54	64.55	-0.01
Hg 4358	75.45	75.46	-0.01

TABLE V
Brucine o-hydroxy-benzoate

Solvent	Chloroform			Pyridine		
Concentration in g./100 c.c.	2.00			0.500		
Calculated	[α]	3.938		9.67		
	[λ]	$\lambda^2 - 0.0915$ 0.3025		$\lambda^2 - 0.0936$ 0.3059		
Line	obs. (α) (0)	cal. (α) (C)	O-C	obs. (α) (0)	cal. (α) (C)	(O-C)
Li 6708	+10.40°	+10.98°	-0.58°	-28.00°	-27.13	+0.87°
Cd 6438	12.23	12.19	+0.04	30.00	30.14	-0.14
Li 6104	13.60	14.02	-0.42	34.50	34.67	-0.17
Na 5893	15.40	15.40	0.00	38.18	38.14	+0.04
Hh 5780	16.80	16.23	+0.57	40.90	40.21	+0.69
Hg 5461	19.50	19.04	+0.46	47.27	47.26	+0.01
Cd 5085	24.09	23.55	+0.54	59.00	58.56	+0.44
Cd 4799	28.60	28.34	+0.26	70.00	70.68	-0.68
Cd 4678	30.90	30.90	0.00	77.18	77.18	0.00
Li 4602	32.22	32.73	-0.51	81.80	81.79	+0.01
Hg 4358	38.80	39.97	-1.17	100.00	100.3	-0.30

The effect of solvent on the Rotatory Power.—The solvent has been shown to profoundly influence the rotatory power of optically active substances. Studies conducted by Pribram⁵ and later workers⁶ indicate that the solvent influences not only the magnitude of but also the direction of rotation.

In the present communication we have described the rotatory powers of brucine salts of six organic acids in various solvents. For the sake of comparison the specific rotatory powers of these salts corresponding to

Hg 5461 line are given in Table I. From a study of this table it will be observed that the sequence of decreasing rotatory power in these solvents is as follows:—

1. *Brucine benzoate*:
Pyridine > Methyl alcohol > Ethyl alcohol > Chloroform.
2. *Brucine-o-amino benzoate*:
Pyridine > Methyl alcohol > Ethyl alcohol > Chloroform.
3. *Brucine-p-amino benzoate*:
Pyridine > Methyl alcohol > Ethyl alcohol > Chloroform.
4. *Brucine-o-hydroxybenzoate*:
Pyridine > Chloroform.
5. *Brucine-m-hydroxybenzoate*:
Pyridine > Methyl alcohol > Ethyl alcohol.
6. *Brucine-p-hydroxy benzoate*:
Pyridine > Methyl alcohol > Ethyl alcohol.

It will be observed that but for brucine-*o*-hydroxy-benzoate the sequence of the decreasing rotatory power runs parallel to the dielectric constants of the solvents except for pyridine. Similar results follow when the comparison is made on the basis of K_D , the rotation constant. However, it will also be noted that all the six brucine salts under examination exhibit the highest rotation in pyridine.

The effect of position isomers on Rotatory Power.—Table I gives the values of the specific rotatory power, $[\alpha]_{D_0}^{20}$, of the compounds under investigation in different solvents. A study of this table shows that in pyridine, where a comparison of the rotatory power of brucine-benzoate and its three hydroxy substitution products is possible, the order of decreasing specific rotatory power is $Un > m > p > o$. This sequence does not agree with Frankland's lever arm hypothesis according to which the effect produced by the *meta* isomer should be intermediate between the *ortho* and *para*. But this order is in full agreement with Cohen's rule⁸ which lays down that the *ortho* substituent has the greatest effect in diminishing or increasing the rotation and that substituents in the *meta* and more especially in the *para* position have a very subordinate effect.

It has not been possible for us to study the rotatory power of brucine-*m*-aminobenzoate as the salt was found to be unstable. It changed at once into a sticky mass. A strict comparison, therefore, of the rotatory powers

TABLE VI
Brucine m-hydroxy-benzoate

Solvent	Ethyl alcohol	Methyl alcohol	Pyridine						
Concentration in gm./100 c.c.	0.500	0.500	0.500						
Calculated $\left\{ \begin{array}{l} (\alpha) \\ (\lambda_0) \end{array} \right\}$	$\frac{6.500}{\lambda^2 - 0.0878}$ 0.2963	$\frac{7.240}{\lambda^2 - 0.0998}$ 0.3159	$\frac{16.07}{\lambda^2 - 0.0949}$ 0.3081						
Line	Obs. (α) (O)	Cal. (α) (C)	Obs. (α) (O)	Cal. (α) (C)	Obs. (α) (O)	Cal. (α) (C)			
Li 6708	-17.27°	-17.95°	-0.68°	-20.00°	-20.67°	-0.67°	-45.40°	-45.27°	+0.13°
Cd 6438	19.00	19.90	-0.90	22.72	23.00	-0.28	50.00	50.29	-0.29
Li 6104	22.70	22.83	-0.13	27.27	26.49	+0.78	57.27	57.90	-0.63
Na 5893	24.54	25.05	-0.51	29.00	29.19	-0.19	63.60	63.17	+0.43
Hg 5780	26.36	26.39	-0.03	30.90	30.89	+0.01	67.27	67.19	+0.08
Hg 5461	30.90	30.89	+0.01	36.36	36.40	-0.04	79.09	79.07	+0.02
Cd 5085	38.18	38.08	+0.10	45.45	45.46	-0.01	98.18	98.13	+0.05
Cd 4799	46.36	45.58	+0.78	55.45	55.46	-0.01	119.00	118.70	+0.30
Cd 4678	50.00	49.58	+0.42	60.90	60.64	+0.26	129.00	129.60	-0.60
Li 4002	52.70	52.86	+0.34	64.54	64.49	+0.05	137.27	137.50	-0.23
Hg 4358	63.60	63.60	0.00	80.00	80.07	-0.07	169.00	169.00	0.00

of brucine benzoate and the three corresponding aminobenzoate is not possible. However, a glance at this table reveals that the ortho substituent has a considerable effect in depressing or enhancing the rotatory power which is again in agreement with Cohen's rule.

The effect of substituent groups on Rotatory Power.—The effect of substituent groups on the rotatory power of optically active compounds has been studied by Rule and other workers. According to Rule⁹ the substituent groups can be arranged on the basis of their polarities and the groups thus arranged follow a definite sequence which is referred to in the literature as "polar series". With minor differences, this general order can be derived from an examination of the benzene substitution data, the influence of different groups on the dissociation constants of substituted acetic or benzoic acids or on the basis of electronic theory and the order is:—

OH⁻, Cl, Br, I, NH₂, C₂H₅, CH₃, H⁺, COOH, CHO, COCH₃, CN.NO₂⁺.

It has been shown that the polar groups have noticeable influence on the rotatory power of an optically active compound and in general the replacement of a hydrogen atom in an optically active compound by a positive substituent displaces the rotation in the opposite sense to that due to a negative substituent. Further, it has been observed that a positive group generally enhances the rotation whereas a negative group depresses it.

Table I gives the values of $[\alpha]_{5461}^{30}$, the specific rotatory power in various solvents. The values of K_0 , the rotation constant, are also given in brackets for the sake of comparison.

Ortho-substitution.—In chloroform the order of decreasing values of $[\alpha]_{5461}^{30}$ and K_0 is H > NH₂ > OH. This sequence shows that the introduction of the electronegative groups decreases the rotatory power and that the order agree with the polar series. In methyl alcohol and ethyl alcohol the values of $[\alpha]_{5461}^{30}$ and K_0 increase with the replacement of H by NH₂. Therefore, in none of these cases the order follows the Rule's generalisation. Comparison of the values of $[\alpha]_{5461}^{30}$ and K_0 in pyridine shows that the order is NH₂ > H > OH. This is in agreement with polar series except for NH₂.

Meta-substitution.—On comparing the values of $[\alpha]_{5461}^{30}$ and K_0 in methyl alcohol, ethyl alcohol and pyridine it will be observed that the introduction of the electronegative OH group depresses the rotation. This is in agreement with the polar series.

Para-substitution.—In chloroform the substitution of H by NH₂ depresses the specific rotatory power. This again agrees with the polar order.

Similar results follow on comparing the values of K_0 , the rotation constant. The order of $[\alpha]_{5461}^{20}$ and K_0 , in methyl alcohol is $\text{NH}_2 > \text{OH} > \text{H}$. This is not in conformity with the polar series. In ethyl alcohol and pyridine the order is $\text{NH}_2 > \text{H} > \text{OH}$. This agrees with Rule's polar series except for NH_2 .

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INFLUENCE OF DIFFERENT PHOSPHATES IN COMPOSTING OF WHEATSTRAW, SAW DUST AND MIXTURES OF SAWDUST WITH STRAW AND DUNG

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IN a previous publication we¹ have shown that on the addition of finely divided phosphate rock or basic slag or superphosphate to plant materials to be composted, there is a greater increase in the fixation of atmospheric nitrogen in the process of composting than in the absence of phosphates. Also, the availability of the phosphate rock or basic slag is increased. In other words, the compost obtained by a mixture of organic matter and calcium phosphate is richer in total nitrogen, available nitrogen and available phosphate than that obtained in the absence of calcium phosphate.

Moreover, we have observed that the compost obtained from straw along with phosphates does not lose nitrogen as readily as those obtained from weeds or water hyacinth and calcium phosphate. It seems that organic substances rich in lignin, when mixed with calcium phosphates, form more stable composts than those obtained from soluble carbohydrates and celluloses mixed with finely divided rock phosphate or basic slag.

We have continued further work on this subject and have studied the composting of straw and sawdust, rich in lignin, under different conditions.

Also, we have studied the carbon-nitrogen transformation of straw mixed with water in presence or absence of small doses of urea kept at a temperature of 35° C.

EXPERIMENTAL PROCEDURE

The composting was carried on in wooden boxes. The joints and openings were closed with a paste to stop any leakage from sides and the bottom. The material to be composted was weighed and then put into the boxes. Samples of materials were previously taken and the dry matter estimated. The requisite amount of soil, *i.e.*, $\frac{1}{3}$ th soil of the total weight of the material was added, mixed well with or without phosphates. The moisture was kept at 60% level. The compost was kept moist throughout the composting period. The temperature was recorded daily and the moisture made up. After composting was continued for a definite period, the whole material

was weighed and then the known weight of the samples was taken out for analysis.

The total carbon was estimated by the method of Robinson, Mclean and Williams and the total nitrogen was estimated by the Kjeldahl method modified by Treadwell and Hall using salicylic acid for reduction of nitrites and nitrates. The percentage analysis is as follows:

Percentage analysis on dry basis

		Sawdust	Soil	Wheatstraw
Total carbon	..	44.90	0.435	30.5
Total nitrogen	..	0.69	0.040	0.618
C/N ratio	..	65	10.8	49.4

The experimental temperatures varied from 20° to 30° C. The results are recorded below:

Composting of sawdust

Period of incubation in days	Total carbon %	Total nitrogen %	Total carbon (gm.)	Total nitrogen (gm.)	Total carbon oxidised (gm.)	Increase of total nitrogen (gm.)	Total carbon oxidised %	Increase of total nitrogen %	C/N ratio
6 Kilograms sawdust + 750 grams soil									
0	39.9	0.617	2697.6	41.7	64
225	35.3	0.699	2153.3	42.63	544.3	0.93	20.1	2.2	50
400	31.8	0.851	1599.3	42.68	1098.3	0.98	40.7	2.3	37
600	29.15	0.941	1314.6	42.43	1383.0	0.73	51.2	1.7	32
2.5 Kilograms wheatstraw + 312.5 grams soil									
0	33.51	0.592	952.5	16.82	56.6
190	26.20	0.980	482.0	18.03	470.5	+1.11	49.3	+ 6.5	26.6
290	20.10	1.240	270.0	16.80	655.5	-0.02	68.8	- 0.11	16.0
2.5 Kilograms wheatstraw + 312.5 grams soil + 14.64 kilograms P_2O_5 as Trichinopoly rock phosphate per ton of organic matter									
0	32.31	0.567	952.5	16.82	56.9
190	26.4	1.400	377.5	20.02	506.1	+3.20	60.3	+19.0	18.8
290	18.6	1.480	239.2	19.15	712.3	+2.33	74.4	+13.8	12.0

Period of incubation in days	Total carbon %	Total nitrogen %	Total carbon (gms.)	Total nitrogen (gms.)	Total carbon oxidised (gms.)	Increase of total nitrogen (gms.)	Total carbon oxidised %	Increase of total nitrogen %	C/N ratio
2.5 Kilograms wheatstraw + 312.5 grams soil + 14.64 kilograms P_2O_5 as Tata basic slag per ton of organic matter									
0	29.81	0.526	952.5	16.82	56.6
190	24.80	1.140	446.4	20.52	506.1	+3.70	53.1	+21.9	21.2
290	17.07	1.440	227.03	19.20	725.5	+2.38	76.1	+14.1	11.7
5 Kilograms sawdust + 750 grams soil + 14.64 kilograms P_2O_5 as Trichinopoly rock phosphate per ton of organic matter									
0	38.5	0.589	2697.6	41.70	65.3
225	33.5	0.730	2137.9	46.58	599.7	4.88	20.7	11.7	45.8
400	30.1	0.910	1542.3	46.63	1155.3	4.93	42.8	11.8	33.0
600	28.20	1.000	1311.8	46.52	1385.8	4.82	51.2	11.5	28.2
6 Kilograms sawdust + 750 grams soil + 14.64 kilograms P_2O_5 as Tata basic slag per ton of organic matter									
0	35.3	0.545	2697.6	41.7	64.7
225	31.2	0.721	2058.4	47.54	639.2	5.84	23.7	14.0	43.2
400	28.8	0.890	1542.2	47.66	1155.4	5.96	42.8	14.3	32.4
600	26.9	1.008	1263.7	47.35	1433.9	5.65	53.1	13.5	26.6

3 Kilograms wheatstraw + 3 kilograms swadust + 750 grams soil						
0	36.20	0.58	2448.6	39.48	..	61.9
225	31.20	0.80	1759.6	45.68	6.20	38.5
400	28.84	0.95	1378.5	45.69	6.21	30.19
600	24.76	0.959	1151.5	44.60	5.12	25.8

When rock phosphate was added to this system, the nitrogen increase was 30°.

3 Kilograms sawdust + 12 kilograms dung + 750 grams soil						
0	28.50	0.895	2198.0	69.31	..	31.7
225	22.41	1.380	1376.8	84.78	15.47	15.0
400	18.40	1.510	990.4	81.22	11.91	12.1
600	17.21	1.510	863.0	77.08	7.71	11.3

In presence of rock phosphate, the increase of total nitrogen went upto 32.5% in these experiments.

A very remarkable fact has been observed by us in these composting experiments. The amount of nitrogen increase by fixation in these composts is always greater when the added soil is low in nitrogen status. Soils rich in nitrogen, when added to the compost, produce small fixation of atmospheric nitrogen.

DISCUSSION

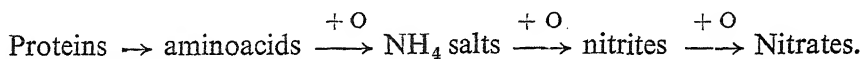
The foregoing experimental results clearly show that in the composting of sawdust rich in lignin mixed with small amounts of soil, there is appreciable fixation of nitrogen in this process. In presence of finely divided rock phosphate or basic slag, the fixation of atmospheric nitrogen is greater than in the absence of phosphates. Moreover, there is fixation of atmospheric nitrogen in the composting of a mixture of dung and sawdust or straw and sawdust. In this case also, the nitrogen fixation is accentuated by adding finely divided rock phosphate or basic slag.

On comparing the results recorded in this communication and those obtained with dung, containing larger amounts or initial total nitrogen recorded in the previous paper, it is observed that there is a tendency to lose the nitrogen of the compost more readily from the dung compost than the compost obtained from straw or sawdust or their mixture. But, the addition of calcium phosphate retards the nitrogen loss from composts.

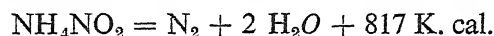
From the balance sheet of carbon and nitrogen, as recorded in the previous tables, one can readily calculate the amount of nitrogen fixed in mgm. per gram of carbon oxidised. It has been observed that the efficiency of the process of nitrogen fixation, *i.e.*, the amount of nitrogen fixed in milligram per gram of carbon oxidised from either sawdust or dung or leaves, singly or in mixtures, is always greater in presence of phosphates. In our experiments on composting the efficiency in absence of phosphates varied from 0.3 to 8.9, whilst in presence of phosphates the efficiency figures were 2.7 to 20.6. When the same energy materials are, however, ploughed in the soil along with phosphates, the nitrogen fixation is still greater. Dhar and Nagpal⁷ have observed that when 200 gm. of a good soil, containing 0.24% total nitrogen and 0.41% P_2O_5 , are mixed with sawdust so that 0.5% carbon is introduced in the system with 25% moisture and allowed to undergo oxidation in air, the amount of nitrogen fixed in mgm. is 80 in light and 41 in the dark per gram of carbon oxidised. In presence of 0.2% P_2O_5 as di-calcium phosphate, these values are 107 in light and 57 in the dark. Moreover, in a calcium phosphate rich soil, the efficiency went up to 150 and in Jumord Sand, as medium the efficiency was 200. Consequently, from the point of view of increase of soil fertility, direct ploughing of plant mate-

rials or dung mixed with phosphates is certainly better than composting of plant materials with phosphates. This point of view has been clearly emphasized by Dhar² in his Presidential Address to the National Academy of Sciences, India, in 1937. It is interesting to note that C/N ratio of the composts obtained in presence of phosphates is smaller than the composts prepared without phosphates.

Under ordinary conditions the proteins present or formed in nitrogen fixation undergo ammonification and nitrification which are also accelerated by light absorption and increase of temperature and form nitrate as in the following scheme:



In these series of reactions, the unstable substance, ammonium nitrite, is formed and it decomposes according to the equation:



Hence, along with nitrogen fixation and formation of proteins and aminoacids due to the oxidation of energy materials both in compost heaps or soils, ammonification and nitrification, which oppose the increase of proteins in the soil or in compost heaps, take place and, thus, the amount of proteins, aminoacids, ammonium salts remaining in the soil or in compost heaps, tends to decrease. Consequently, the apparent efficiency of the fixation of nitrogen, *i.e.*, the amount of nitrogen fixed in milligram per gram of carbon of the organic matter oxidised, falls off when ammonification and nitrification take place. But, in presence of large amounts of phosphates in the system, more or less stable phospho-proteins are formed by the combination of proteins and phosphorous compounds. These compounds seem to resist ammonification, nitrification and loss of nitrogen better than proteins alone. This appears to be an important reason why the efficiency of nitrogen fixation in soils or in compost heaps appears to be larger in presence of increasing quantities of phosphates.

In the following tables some of our results on nitrogen fixation and nitrogen conservation in composting have been summarised. The results show that nitrogen conservation in the composting of quickly decomposing fresh green materials like weeds and water hyacinth is appreciably greater in presence of phosphates than in their absence. Similarly, in composting dung, straw, sawdust containing lignin, the nitrogen fixation in presence of phosphates is appreciably greater than in their absence.

Composting experiments				Initial C/N ratio	Final nitrogen % (Dry basis)	Nitrogen conservation %
1.	Weeds + Soil	12.7	0.700	43.2
2.	Water hyacinth alone	17.6	2.500	54.5
3.	Cowdung + Soil	27.9	1.110	102.5
4.	Wheat straw + Soil	49.6	1.070	110.5
5.	Wheat straw + Soil	56.6	0.950	106.5
6.	Sawdust + Straw + Soil	61.9	1.120	115.7
7.	Sawdust + Straw + Soil	114.7	0.590	117.8

The same materials were used in the above Table when mixed with Phosphates.

Composting experiments				Initial C/N ratio	Final nitrogen % (Dry basis)	Nitrogen conservation %
1.	Weeds + Rock phosphate (Trichinopoly)	12.7	0.800	44.8
2.	Water hyacinth + Rock phosphate (Trichinopoly)	17.6	2.850	64.9
3.	Cowdung + Rock phosphate (Trichinopoly)	17.9	1.180	105.7
4.	Wheat straw + Rock phosphate (Trichinopoly)	49.4	1.183	129.2
5.	Wheat straw + Rock phosphate (Trichinopoly)	56.6	1.400	119.0
6.	Sawdust + Straw + Rock phosphate (Trichinopoly)	61.9	1.120	129.9
7.	Sawdust + Straw + Algerian rock phosphate	114.7	0.835	130.9

Composting experiments	Initial C/N ratio	Final nitrogen % (Dry basis)	Nitrogen conservation %
1. Weeds + Superphosphate ..	12.7	1.050	43.3
2. Water hyacinth + Tata basic slag	17.6	2.550	66.4
3. Cowdung + Tata basic slag ..	17.9	1.200	166.8
4. Wheat straw + Superphosphate + Rock phosphate	49.4	1.200	138.2
5. Wheat straw + Tata basic slag	56.6	1.140	121.9
6. Straw + Sawdust + Tata basic slag	61.9	0.880	135.7
7. Straw + Sawdust + Algerian rock phosphate	114.7	0.875	130.5

NITROGEN FIXATION IN THE INCUBATION OF STRAW

In some interesting experiments in the incubation of straw at 36° C. or 26° C., Hutchinson and Richards, in their researches on the preparation of artificial farm-yard manure, recorded the following observations:

Total Nitrogen

	Tempe- rature	Loss of dry matter	Initial (mgm.)	Final (mgm.)	Effici- ency	Loss (—) or gain (+)
Straw with water ..	36° C.	40.1	71	97	16	+ 26
(I) Straw with urine ..	26° C.	49.1	507	178	..	—329
Straw with urine ..	36° C.	59.8	507	176	..	—331

		Number of experiments				
		1	2	3	4	5
At beginning—Total Nitrogen ..	77.5	157.0	237.6	317.6	397.6	
(II) Straw and urine nitrogen after 86 days	77.3	153.1	226.8	262.1	308.0	

(III) *The decomposition of straw in presence of varying quantities of nitrogen as urea*

	Number of experiments									
	1	2	3	4	5	6	7	8	9	10
Straw Nitrogen	..	71	71	71	71	71	71	71	71	71
Urea Nitrogen (mgm.)	..	0	5	10	24	48	97	243	486	729
Total Nitrogen	..	71	76	81	95	119	168	314	557	800
After 3 months organic Nitrogen (mgm.)	..	180	177	174	190	192	171	245	269	181
Ammonia Nitrogen	..	0	5	2	4	4	29	74	68	71
Total	..	180	182	176	194	196	200	319	337	252
Gain or loss	..	+109	+106	+95	+99	+77	+32	+5	-220	-558
Dry matter loss %	..	49	46	45	49	47	53	51	48	19
Efficiency calculated by us, <i>i.e.</i> , Nitrogen fixed in milligrams per gram of Carbon oxidised	..	57	58	67	52	42	15	2.5

The efficiency recorded above is very high and is much higher than observed in series No. (I), *i.e.*, 16. We have not so far obtained such high fixation in our experiments in composting or incubation of straw, but higher fixations have been obtained by us in our nitrogen fixation experiments, specially in presence of sunlight and calcium phosphates by ploughing in organic matter in soil or incorporating organic matter in soil or sand in dishes.

In discussing the observations recorded in series No. (I), the authors stated as follows:

"The three equal portions of straw were saturated either with water or urine and allowed to ferment for 3 months in the laboratory, the two portions with urine being subjected to different temperatures. As will be seen from the foregoing table, these two portions fermented to different degrees—the dry matter losses being 49 and 60% respectively but the final nitrogen content was almost identical and practically $\frac{3}{4}$ th of the nitrogen supplied as urine was lost."

Regarding the results recorded in series No. (II), Hutchinson and Richards have stated as follows:

"The above results show that losses of nitrogen occurring after satisfactory rotting do not exceed 4% in the lower concentrations of nitrogen. The ordinary losses of the manure heaps are frequently more than tenfold this amount."

Discussing the results recorded in series No. (III), the authors reported as follows:

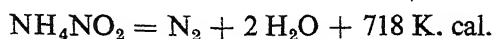
"Ten portions of straw were moistened to the same extent and while one received water only, the others received additions of soluble nitrogen in the form of urea in varying quantities until the last portion was saturated with a solution similar in concentration of nitrogen to that of horse urine (1% nitrogen). The different portions were kept in an incubator for 3 months, at the end of which time it was evident that contrary to expectation, the straw without or merely with low doses of nitrogen, had passed through a marked rotting process. On analysis it was found that there had been a definite accumulation of nitrogen in the lower members of the series, while the higher members had lost in some cases the greater portion of their original nitrogen. In seven out of the ten cases the final nitrogen of the fermented straw varied only between 180 to 210 mgm. irrespective of the nitrogen content of the original mixture. It should also be noted that the extent of the rotting, *i.e.*, loss of dry matter, in experiments 1-8 was very

much greater than in 9 and 10 in which the straw was subjected to the action of solutions closely approaching the concentration of ordinary urine, the high alkalinity of the latter exercising a check on decomposition. In the main, the nitrogen retained by supersaturated straw or such as is accumulated by undersaturated straw, as in Nos. 1-6 in the above table, appears to be stored up in organic or non-ammoniacal form. The maximum retention has been found to occur within the first 4 weeks, after which time breakdown of this organic nitrogen to ammonia and consequent loss by volatilisation seems to keep pace with loss of dry matter. Finally, the material assumes a "stabilised" condition—the loss of nitrogen becomes greatly diminished or may be absent all together for long periods. It may be stated that when straw has worked from an unsaturated to a stable phase little or no free ammonia is to be found, but straw which commences with a super-abundance of nitrogen appears to hold when in a fermented state, upwards of 14% of its nitrogen in the form of ammonia so long as the material is in a moist condition. Desiccation leads almost to complete loss of ammonia and in this respect as well as in the proportion of ammonia in the moist material, the artificial resembles the natural manure. From the study of the interrelations between nitrogen and straw, we have come to the conclusion that the amount of nitrogen necessary for pronounced rotting, the amount which straw is capable of fixing in the form of ammonia are identical and that in general the figure varies only between 0.70 and 0.75 parts of nitrogen per 100 parts of dry straw."

From the foregoing quotations it is quite clear that Hutchinson and Richards have not been able to explain at all the increase in the nitrogen content of straw moistened with water alone or containing small amount of urea. The most interesting point is that the carbonaceous matter present in the straw has undergone marked loss to the extent of 40% in the series No. (I) and 49% in the series No. (III) even in the complete absence of urine or urea. There is no doubt this loss of organic matter has been caused by the oxidation of the cellulose and other carbonaceous compounds. Dhar was the first to explain this increase of nitrogen with straw alone from the view-point of atmospheric nitrogen fixation caused by the liberation of energy evolved in the slow oxidation of cellulose and lignin of straw. This oxidation process has liberated energy, which has been actually utilised in fixing nitrogen by straw to the extent of 26 mgm. in series No. (I) and 109 mgm. in series No. (III) in the complete absence of the additional nitrogenous substances. Increase of nitrogen varying from 106 mgm. upto 5 mgm. have taken place in presence of amounts of urea containing 5 to 243 mgm. nitro-

gen. These results are also due to the fixation of atmospheric nitrogen in the process of the loss of dry matter varying from 46 to 51%.

On the other hand, when the added urine or urea is in large concentrations, marked nitrification of the urea takes place and in this process the unstable substance, ammonium nitrite, is formed and decomposes according to the equation:



This is certainly the most important cause of the losses of nitrogen recorded in the experiments of Hutchinson and Richards. There may be a small loss of nitrogen as ammonia gas.

We have repeated some of the experiments in the incubation of straw and our results are recorded below:—

Analysis of wheat straw

Total Carbon	.. 36.60%
Total Nitrogen	.. 0.616% C/N ratio: 59.4
Total dry matter	.. 11.4 gm.
Incubated at 35° C. for 3 months	

		Initial total Nitrogen (mgm.)	Total Nitrogen after 3 months %	Total Nitrogen after 3 months (mgm.)	Increase in Nitrogen (mgm.)	Loss of Dry matter %
1. Straw + Water	..	66.53	1.93	91.55	25.02	41.6
2. Straw + 5 mgm. Urea Nitrogen		71.53	2.10	93.76	22.23	39.1
3. Straw + 10 mgm. Urea Nitrogen		76.53	1.80	92.98	16.45	45.0
4. Straw + 24 mgm. Urea Nitrogen		90.53	2.30	97.92	6.39	36.1

These experiments show that in every case marked fixation of nitrogen takes place at 35° C. after incubation for 3 months. The efficiency of nitrogen fixation in No. 1 is 14 whilst Hutchinson and Richards' value as calculated by us is 16.

From our experiments recorded in the foregoing pages and the above discussion, it is clear that for the preparation of stable composts both calcium phosphate and lignin are useful, and such composts can be prepared from straw, dung and sawdust and calcium phosphates with fixation of atmospheric nitrogen. Green materials when incorporated in the soil mixed with calcium phosphates can readily fix atmospheric nitrogen but cannot build up humus permanently.

SUMMARY

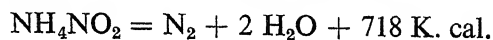
A rapid rise in the temperature of the mixture was observed in the beginning of composting with a slow downward gradient afterwards. In composting of wheat straw, sawdust rich in lignin or mixtures of sawdust and straw or sawdust and dung, when mixed with small amounts of soil, there is an appreciable fixation of nitrogen from air.

In presence of finely divided rock phosphate or basic slag (32.4 lb. P_2O_5 per ton of organic matter), the fixation of nitrogen is greater than in absence of phosphates.

It has been observed that the efficiency of the process of nitrogen fixation, *i.e.*, the amount of nitrogen fixed in milligrams per gram of carbon oxidised, of straw or sawdust, singly or in mixtures, is always greater in presence of phosphates. Under ordinary conditions the proteins present or formed in nitrogen fixation undergo ammonification and nitrification, which oppose the increase of proteins in compost heaps and, thus, the amount of proteins remaining in compost heaps, tend to decrease. Consequently, the apparent efficiency falls off.

It is interesting to note that the C/N ratio of the compost obtained in presence of phosphates is lower than of the compost prepared without phosphates.

The loss of nitrogen is due to the high temperature prevailing in the compost heaps and acidity produced during the decomposition of organic matter in heaps. These lead to the rapid decomposition of the unstable substance, ammonium nitrite, formed in the nitrification of nitrogenous compounds, according to the following equation:



The loss of nitrogen can be minimised if the temperature of the system and its acidity are not allowed to rise much. This can be achieved by turning the mass. The temperature in these experiments did not exceed 37° C.,

whilst, in the majority of methods of composting, the temperature rises beyond 70° C. leading to marked loss of nitrogen.

Experimental results show that incubation of straw mixed with 50% of its weight of water in glass beakers for 3 months leads to a fixation of atmospheric nitrogen of 37.7% of the total nitrogen present in the straw.

Even in presence of small amount of urea, there is also appreciable fixation of atmospheric nitrogen in these incubation experiments of straw. The amount of fixation decreases as the concentration of urea increases. Similar increase in nitrogen is recorded in the experiments of Hutchinson and Richards in Rothamsted.

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EXAMINATION OF THE SOLUBILITY DATA FOR
THE CALCULATION OF COMPOSITION,
FORMATION CONSTANT AND FREE ENERGY,
ENTHALPY AND ENTROPY CHANGES IN THE
FORMATION OF SILVER-AMMONIA
COMPLEX FORMED IN THE DISSOLUTION OF
SILVER BROMIDE IN AQUEOUS AMMONIA

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ABSTRACT

The data of Böldlander and Fittig on the solubility of silver bromide in aqueous ammonia have been used to calculate the composition of the complex ammino-silver bromide formed, by the method described earlier. The stoichiometry of the components in the complex is 1:1. The data have further been utilised to calculate the formation constant and $\log K$ found to be 2.35 and 2.74 respectively at 15° and 25° C. The free energy of formation works out to be -3.26 C cal. at 15° C. The values of ΔH° and ΔS° have been calculated to be -15.6 K cal. and -42.9 cal./degree mole respectively.

A NEW treatment of solubility data to elucidate the composition of a soluble complex by dissolution of a sparingly soluble substance in a solution has been given by Dey.¹ He employed this calculation successfully to several complex forming systems, viz., to (i) polyhalide formation in solutions of iodine and bromine in potassium iodide and bromide respectively,² (ii) polychloride formation in the dissolution of chlorine in hydrochloric acid,³ and (iii) formation of complex argentothiosulphates in solutions of silver halides in sodium thiosulphate.^{4,5}

In previous papers^{6,7} we have made an attempt to extend these calculations and have been able to work out methods to utilise the solubility data for the calculation of formation constant, standard free energy change, standard heat content or enthalpy change and the standard entropy change in the formation of complex ions in solution.

Böldlander and Fittig^{8,9} determined the solubility of silver bromide in aqueous ammonia at two different temperatures. In this paper, we have used their values, to calculate the composition of the complex ions formed

in solution. We have also calculated the values of K , ΔF° , ΔH° and ΔS° from these data.

The reaction between silver bromide and ammonia may be represented by:



By the law of mass action:

$$\frac{[\text{Complex}]}{[\text{NH}_3]^n [\text{AgBr}]} = K.$$

If a and b are the solubility of silver bromide in water and ammonia solution of concentration c respectively, then at equilibrium,

$$[\text{Complex}] = (b - a)$$

$$[\text{AgBr}] = a$$

$$[\text{NH}_3]_{\text{used}} = n(b - a)$$

$$[\text{NH}_3]_{\text{free}} = c - n(b - a)$$

Therefore,

$$\frac{(b - a)}{(c - bn + an)^n \cdot a} = K.$$

and for other corresponding concentrations a' , b' and c' ,

$$\frac{(b' - a')}{(c' - b'n + a'n)^n \cdot a'} = K.$$

Let $(b - a) = s$ and $(b' - a') = s'$, and since $a' = a$,

$$\frac{s}{(c - sn)^n \cdot a} = \frac{s'}{(c' - s'n)^n \cdot a}$$

or

$$\frac{s}{s'} = \left[\frac{(c - sn)}{(c' - s'n)} \right]^n.$$

Taking logarithms of both sides we have,

$$\log \frac{s}{s'} = n [\log (c - sn) - \log (c' - s'n)].$$

Expanding the right-hand side in power series and neglecting the second and higher terms of the series (since s is small in comparison to c) we finally get,

$$\log s/s' = n \log c/c'$$

or

$$n = \frac{\log s/s'}{\log c/c'}$$

$$= \frac{\log (b-a)/(b'-a)}{\log c/c'}$$

Since again, a is negligible for silver bromide, in comparison to b , the expression finally becomes,

$$n = \frac{\log b/b'}{\log c/c'}$$

With the help of the above expression, we have calculated the value of n and K from the data given below.

TABLE I
Solubility of silver bromide in aqueous ammonia at 15° C.
(Data from Böldander⁸)

Concentration of silver bromide dissolved gmM./litre	Concentration of ammonia gmM./litre	Value of n	Log K (for $n = 1$)
0.0011	1.0850	..	2.23
0.0031	2.3650	1.3	2.34
0.0050	3.4100	1.3	2.39
0.0074	4.9500	1.3	2.33
0.0101	5.725	1.4	2.47
		Average log $K = 2.35$	

From the tables I & II it may be seen that the value of n is approximately unity, showing that the composition of the complex ion is $[\text{Ag}(\text{NH}_3)]^+$. This is further corroborated by the constancy of $\log K$, when calculated with $n = 1$.

The values of ΔF° , ΔH° and ΔS° have been calculated with the help of the relations given below:

$$\Delta F^\circ = -RT \ln K$$

$$\frac{d \ln K}{dT} = \frac{\Delta H^\circ}{RT^2}$$

$$\Delta F^\circ = \Delta H^\circ - T\Delta S^\circ$$

TABLE II

Solubility of silver bromide in aqueous ammonia at 25° C.(Data from Böldlander and Fittig⁹)

Concentration of silver bromide dissolved gmM./litre	Concentration of ammonia gmM./litre	Value of n	Log K (for $n = 1$)
0.0006	0.1932	..	2.71
0.0012	0.3849	1.0	2.72
0.0022	0.7573	0.9	2.69
0.0069	1.9650	1.2	2.77
0.0116	3.0240	1.2	2.81
0.0244	5.2440	1.3	
Average log $K = 2.74$			

where

 ΔF° = the standard free energy change, R = Gas constant, T = Absolute temperature, K = Formation constant, ΔH° = Standard heat content change,

and

 ΔS° = Standard entropy change.

In the system studied here, the values of ΔF° , ΔH° and ΔS° work out respectively to be -3.26 K. cals., -15.6 K. cals. and -42.9 cals.

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part V. Effect of *p*-Aminobenzoic Acid on Yeast Growth

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Received on October 19, 1956

THE study of microbiological growth factors was started at the beginning of the 19th century when for the first time Wildiers¹ put forward his conception of 'bios'. Bios was a certain hypothetical substance of biological origin which Wildiers considered essential for the growth of yeasts. Bios has been found now to be a complicated substance consisting of several active factors, such as meso-inositol,² pantothenic acid,³ biotin,⁴ etc.

p-Aminobenzoic acid is believed to stimulate the growth of certain yeasts.⁵ Very little literature is available on the individual effect of this substance on the growth of yeasts. Cutts and Rainbow⁶ have found that growth of certain yeasts is retarded in the presence of *p*-aminobenzoic acid. A particular strain, *Saccharomyces cerevisiae* '47', required a concentration of 0.007 gm. per c.c. of medium for half-maximum growth. Adenine can replace *p*-aminobenzoic acid if methionine alone, or better if methionine and histidine both, are present in the medium. The growth-promoting activity of *p*-aminobenzoic acid is said to increase in the presence of amino-acids, such as methionine, histidine, leucine, norleucine, valine, tyrosine, etc.⁷

We have attempted to find the influence of different concentrations of *p*-aminobenzoic acid on *Dhar yeast*, *Sacchchromyces cerevisiae*, *Torulopsis utilis* and *Rhodotorula gracilis*. We were more interested in *Dhar yeast*⁸ which has been isolated in our laboratory from the palm toddy of Allahabad, by Dhar and Bahadur. *Dhar yeast* is a new strain of pichia genus and has several remarkable features.⁹

EXPERIMENTAL

100 c.c. of the following medium, the pH of which was adjusted to 4.6, was taken in several 250 c.c. conical flasks. Different amounts of *p*-aminobenzoic acid were added to the flasks:—

Glucose 5.00 gm.
Ammonium sulphate 0.4000 gm.
Potassium dihydrogen phosphate 0.1530 gm.

Potassium sulphate 0.3020 gm.
Magnesium sulphate 0.0500 gm.
Calcium chloride 0.0100 gm.
Ferrous sulphate.. 0.0017 gm.
Cupric sulphate 4 μ gm.
Zinc sulphate 50 μ gm.

Distilled water to make 100 c.c.

The flasks were sterilized at 10 lb. pressure for 30 minutes and seeded with a drop of fresh yeast suspension containing negligible amount of yeast. Temperature of incubation varied between 33° C. and 36° C. *Dhar yeast* and *R. gracilis* were incubated for 3 weeks while *S. cerevisiae* and *T. utilis* were incubated for 2 weeks. After incubation, yeasts were filtered and washed through gravimetric filter-papers (Whatman No. 42) under gentle suction. Unconsumed glucose was estimated in the filtrate by Fehlings Solution with methylene blue as indicator. Yeasts were dried for about 10 hours at 75° C. to 80° C. to a constant weight.

TABLE I
Dhar yeast

<i>p</i> -Aminobenzoic acid added (μ gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven- dry yeast (gm.)	Yield of yeast on fermented sugar %
0	5.000	1.515	3.485	0.704	20.2
10	5.000	1.486	3.514	0.766	21.8
20	5.000	1.442	3.558	0.850	23.9
30	5.000	1.435	3.565	0.902	25.3
40	5.000	1.428	3.572	0.930	26.0
50	5.000	1.433	3.567	0.926	26.0

TABLE II

Rhodotorula gracilis

<i>p</i> -Aminobenzoic acid added (μgm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven-dry yeast (gm.)	Yield of yeast on fermented sugar %
0	5.000	3.884	1.116	0.452	40.5
10	5.000	3.884	1.116	0.452	40.5
20	5.000	3.885	1.115	0.452	40.5
30	5.000	3.885	1.115	0.451	40.5
40	5.000	3.886	1.114	0.450	40.4
60	5.000	3.887	1.113	0.450	40.4
80	5.000	3.890	1.110	0.448	40.3

TABLE III

Saccharomyces cerevisiae

<i>p</i> -Aminobenzoic acid added (μgm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven-dry yeast (gm.)	Yield of yeast on fermented sugar %
0	5.000	Nil	5.000	0.096	1.9
10	5.000	„	5.000	0.110	2.2
20	5.000	„	5.000	0.119	2.4
30	5.000	„	5.000	0.121	2.4
40	5.000	„	5.000	0.121	2.4
50	5.000	„	5.000	0.120	2.4

TABLE IV
Torulopsis utilis

<i>p</i> -Aminobenzoic acid added (μgm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven-dry yeast (gm.)	Yield of yeast on fermented sugar %
0	5.000	Nil	5.000	0.146	2.9
10	5.000	„	5.000	0.237	4.7
20	5.000	„	5.000	0.287	5.7
30	5.000	„	5.000	0.323	6.5
40	5.000	„	5.000	0.344	6.9
50	5.000	„	5.000	0.338	6.8

DISCUSSION

From our results it appears that *p*-aminobenzoic acid (PABA) has small growth-stimulating action on *Dhar yeast* and *T. utilis*. It has only a slight growth-promoting effect over *S. cerevisiae* while the growth of *R. gracilis* is retarded by it. In case of *Dhar yeast*, PABA slightly increases the rate of fermentation of glucose. In all cases where yeast yield is increased, increasing quantities of PABA regularly increase the yields of yeasts until after the optimum cocentration of PABA they are slightly decreased.

PABA, thus, is slightly favourable to the growth of *Dhar yeast* and *T. utilis*. It does not possess appreciable growth-promoting action on *S. cerevisiae* and in case of *R. gracilis* the growth is slowly retarded.

SUMMARY

Different concentrations of *p*-aminobenzoic acid were tried to study its growth-promoting effect on *Dhar yeast*, *Rhodotorula gracilis*, *Saccharomyces cerevisiae* and *Torulopsis utilis*. It showed slight growth-promoting action over *Dhar yeast* and *T. utilis*. It had no significant effect on the growth of *S. cerevisiae* while it retarded the growth of *R. gracilis*.

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part VI. Effect of Aneurin Hydrochloride (Vitamin B₁) on Yeast Growth

BY N. R. DHAR, S. P. MITRA AND K. VADALKAR

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Received on October 23, 1956

ANEURIN is one of the important growth factors which is believed to possess growth-promoting influence over many yeasts. Farrel¹ designated the name 'bios V' to a substance which was responsible to stimulate the growth of *Saccharomyces hansenospora valbyensis*. The latter was found to grow well in tomato juice but failed to grow satisfactorily in a medium containing biotin, inositol or pantothenic acid. Farrel, as such, thought of some new growth factor which she named as 'bios V'. Two years later, it was shown by Miller² that 'bios V' can be replaced by aneurin.

Aneurin is closely related to cocarboxylase and is believed to function in the structure of the latter compound. It is more useful as a co-factor which reinforces the growth-promoting influence of bios and other growth-promoting factors. Williams and Saunders³ have found that small amounts of aneurin, when added with pantothenic acid, appreciably increase the growth of certain yeasts. Schultz, Atkin and Frey⁴ have found that this vitamin stimulates also the rate of fermentation of certain Baker's yeast. Williams and Rohem⁵ had also evidence of growth-promoting influence of aneurin on some yeasts. Wickerham⁶ found that aneurin with other growth factors helps yeasts to assimilate different sources of nitrogen which they are unable to utilize otherwise.

We have attempted to investigate the influence of different concentrations of aneurin hydrochloride on the growth of *Dhar yeast*,⁷ *Rhodotorula gracilis*, *Saccharomyces cerevisiae* and *Torulopsis utilis*.

EXPERIMENTAL

100 c.c. of the following medium, the pH of which was adjusted to 4.6, was taken in several 250 c.c. conical flasks. Different quantities of aneurin hydrochloride were added to the flasks:

Glucose	5.00 gm.
Ammonium sulphate	0.4000 gm.
Potassium dihydrogenphosphate	0.1530 gm.

Potassium sulphate	0.3020 gm.
Magnesium sulphate	0.0500 gm.
Calcium chloride	0.0100 gm.
Ferrous sulphate	0.0017 gm.
Cupric sulphate	4 μ gm.
Zinc sulphate-.....	50 μ gm.

Distilled water to make 100 c.c.

The flasks were sterilized at 10 lb. pressure for 30 minutes and seeded with a drop of fresh yeast suspension containing negligible amount of yeast. Temperature of incubation varied between 33° C. and 35° C. *Dhar yeast* and *R. gracilis* were incubated for 3 weeks while *S. cerevisiae* and *T. utilis* were incubated for 2 weeks. After incubation, the yeasts were filtered and washed with distilled water through gravimetric filter-papers (Whatman No. 42) under gentle suction. Unconsumed glucose in the filtrate was estimated by Fehling's solution with methylene blue as indicator. The yeasts were dried for about 10 hours at 75° C. to 80° C. to constant weight.

TABLE I
Dhar yeast

Aneurin hydrochloride added (μ gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven- dry yeast (gm.)	Yield of yeast on fermented sugar (%)
0	5.000	1.750	3.250	0.699	21.5
10	5.000	1.777	3.223	0.706	21.9
20	5.000	1.811	3.189	0.719	22.6
30	5.000	1.877	3.123	0.735	23.5
40	5.000	1.924	3.076	0.748	24.3
60	5.000	1.951	3.049	0.758	24.8
80	5.000	1.955	3.045	0.756	24.8

TABLE II

Rhodotorula gracilis

Aneurin hydrochloride added (μ gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven-dry yeast (gm.)	Yield of yeast on fermented sugar (%)
0	5.000	3.829	1.171	0.474	40.5
10	5.000	3.814	1.186	0.497	41.9
20	5.000	3.800	1.200	0.512	42.6
30	5.000	3.770	1.230	0.533	43.4
40	5.000	3.552	1.448	0.643	44.4
50	5.000	3.555	1.445	0.640	44.4

TABLE III

Saccharomyces cerevisiae

Aneurin hydrochloride added (μ gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven-dry yeast (gm.)	Yield of yeast on fermented sugar (%)
0	5.000	Nil	5.000	0.099	2.0
10	5.000	„	5.000	0.156	3.1
20	5.000	„	5.000	0.201	4.0
30	5.000	„	5.000	0.233	4.6
40	5.000	„	5.000	0.255	5.1
50	5.000	„	5.000	0.266	5.3
60	5.000	„	5.000	0.262	5.2

TABLE IV

Torulopsis utilis

Aneurin hydrochloride added (μ gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven-dry yeast (gm.)	Yield of yeast on fermented sugar (%)
0	5.000	Nil	5.000	0.117	2.3
10	5.000	„	5.000	0.130	2.6
20	5.000	„	5.000	0.138	2.7
30	5.000	„	5.000	0.146	2.9
40	5.000	„	5.000	0.160	3.2
50	5.000	„	5.000	0.179	3.6
60	5.000	„	5.000	0.178	3.5

DISCUSSION

Our results indicate that aneurin has some definite, though small, growth influence on *Dhar yeast*, *R. gracilis* and *S. cerevisiae*. The growth of *T. utilis* is only slightly stimulated by this vitamin. The rate of fermentation of glucose is gradually increased with the increasing quantities of aneurin in cultures of *Dhar yeast* while with *R. gracilis* the amounts of glucose fermented are very slightly decreased. In the cultures of *S. cerevisiae* and *T. utilis*, all the sugar is consumed with very little increase in yeast yield.

Aneurin, thus, seems to possess a small growth-promoting influence over *Dhar yeast*, *R. gracilis* and *S. cerevisiae*. It does not affect significantly the growth of *T. utilis*.

SUMMARY

Different concentrations of aneurin hydrochloride were taken in synthetic media devoid of vitamins and growth substances to study its influence on the growth of *Dhar yeast*, *Rhodotorula gracilis*, *Torulopsis utilis* and *Saccharomyces cerevisiae*. It showed a small growth-promoting influence

over *Dhar yeast*, *R. gracilis* and *S. cerevisiæ*. The growth of *T. utilis* was not appreciably influenced by it.

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Although the exact mechanism of alcoholic fermentation is yet a mystery there is a good deal of evidence in support of Mayerhof's scheme. Parnas⁶ and others have demonstrated the role of adenosine triphosphate and adenylic acid as phosphorylating and dephosphorylating reagents respectively. The intermediates postulated by Mayerhof, *i.e.*, 3-glyceraldehydephosphate and dihydroxy acetone phosphate have been isolated from yeast extract and also synthesised by Fischer.⁷

We have attempted to investigate the influence of different concentrations of potassium dihydrogen phosphate over yeast growth and alcohol production in cultures of *Dhar yeast*⁸ which has recently been isolated in our laboratory.

EXPERIMENTAL

200 c.c. of the following medium, the pH of which was adjusted to 4·6, was taken in several 500 c.c. conical flasks. Different quantities of Merck grade dry potassium dihydrogen-phosphate were added to different flasks.

Glucose	10·00 gm.
Ammonium sulphate	0·8000 gm.
Potassium sulphate	0·6040 gm.
Magnesium sulphate	0·1000 gm.
Calcium chloride	0·0200 gm.
Ferrous sulphate	0·0034 gm.
Cupric sulphate	8 microgram
Zinc sulphate	100 microgram

Distilled water to make 200 c.c.

The flasks were sterilized at 15 lb. pressure for 20 minutes and seeded with a drop of fresh yeast suspension containing negligible amount of yeast. The flasks were kept in an incubator at 33° to 35° C. for 28 days. After incubation yeast was filtered and washed with distilled water through Whatman No. 50 filter-paper under suction. Unconsumed glucose in the filtrate was estimated by Fehling's solution with methylene blue as indicator. Amount of alcohol in the filtrate were estimated by distilling it and finding the density by pyknometer.⁹ Yeast was dried at 100 to 105 ° C. in a oven to a constant weight for about 5 to 6 hours.

TABLE I

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Yield of oven- dry yeast (gm.)	Yield of yeast on fermented glucose (%)
Nil	10.00	10.00	Nil	Nil	Nil
0.100	10.00	5.739	4.261	0.750	13.0
0.200	10.00	4.673	5.327	0.831	15.6
0.300	10.00	4.120	5.880	1.106	18.8
0.400	10.00	3.823	6.177	1.260	20.4
0.500	10.00	3.012	6.988	1.342	19.2
0.600	10.00	2.291	7.709	1.411	18.3
0.700	10.00	2.204	7.796	1.402	18.0

TABLE II

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose fermented (gm.)	Alcohol produced (gm.)	Yield of Alcohol fermented on glucose (%)
Nil	10.00	10.00	Nil	Nil	Nil
0.100	10.00	5.739	4.261	"	"
0.200	10.00	4.673	5.327	0.201	3.8
0.300	10.00	4.120	5.880	0.400	6.8
0.400	10.00	3.823	6.177	0.600	9.7
0.500	10.00	3.012	6.988	0.400	5.7
0.600	10.00	2.291	7.709	Nil	Nil
0.700	10.00	2.204	7.796	"	"

DISCUSSION

From our results it is clear that without phosphate growth of *Dhar yeast* is not possible. The production of yeast is gradually increased with increasing doses of potassium dihydrogen phosphate until at a concentration of 0.35 per cent., the growth of yeast becomes maximum. However, at a concentration of 0.2, the efficiency of yeast formation, *i.e.*, the amount of yeast obtained by the complete fermentation of 100 grams of glucose, is maximum. With increasing concentration of the phosphate, amounts of fermented glucose regularly increase.

Very little alcohol is formed in *Dhar yeast* cultures and very low and very high doses of the phosphate do not form any alcohol. This may be attributed to the oxidation of alcohol formed because *Dhar yeast* has been found to utilize alcohol as source of energy.¹⁰

SUMMARY

Different concentrations of potassium dihydrogen phosphate were attempted to see its influence on the growth of yeast and production of alcohol in *Dhar yeast* cultures. At a concentration of 0.2 per cent., the yield of yeast on fermented glucose was maximum although the actual yield of yeast was maximum at a concentration of 0.35 per cent.

Fermentation of glucose was increased regularly with the increase in the concentration of phosphate. Formation of alcohol was slightly increased by the phosphate. Concentrations, higher than 2.5 per cent., led to the formation of no alcohol.

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part VIII. Effect of Different Concentrations of Potassium Dihydrogen Phosphate on the Carbon and Nitrogen Balance in *Dhar* yeast Cultures

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PHOSPHORUS is an integral part of yeast cells and so indispensable to yeast life. Phosphate is also indispensable in the fermentation of sugar into alcohol by yeasts. Harden and Young¹ found that without a phosphate, the fermentation of glucose into alcohol by yeast extract was not possible. Neuberg² and Kluyver³ pushed further the study of the role of phosphates in the fermentation of sugars by yeasts. Meyerhof⁴ gave a scheme which elucidates the role of the phosphate in the mechanism of fermentation of sugars into alcohol by yeasts. The scheme depicts the various intermediate stages in the conversion of glucose into alcohol. The scheme has been supported by the works of Parnas,⁵ Fischer⁶ and others, although the exact mechanism is yet an unsolved problem. Still more important is the part played by phosphates in the growth of yeast and its various metabolic activities.

We have attempted to study the influence of different concentrations of potassium dihydrogen phosphate on the carbon balance and nitrogen balance in *Dhar* yeast⁷ cultures. We have also studied the variations in the percentage of carbon, nitrogen and C/N ratio in yeast with different concentrations of potassium dihydrogen phosphate.

EXPERIMENTAL

200 c.c. of the following medium, of which the pH was adjusted to 4.6, was taken in several 500 c.c. conical flasks and different quantities of dry Merck grade potassium dihydrogen phosphate were added to them.

Glucose	10.00	gm.
Ammonium sulphate	0.8000	gm.
Potassium sulphate	0.6040	gm.
Magnesium sulphate	0.1000	gm.
Calcium chloride	0.0200	gm.

TABLE I
Carbon balance

KH ₂ PO ₄ added (gm.)	Yield of oven-dry yeast (gm.)	Carbon ingoing			Carbon outgoing			
		Glucose (gm.)	Seed yeast (gm.)	Total (gm.)	Net yeast (gm.)	Spent media (gm.)	Carbon loss (gm.)	Total (gm.)
0.100	0.7502	3.9452	negligible	3.9452	0.3128	0.6241	3.0083	3.945
0.200	0.8311	3.9452	"	3.9452	0.3799	0.4941	3.0712	3.945
0.300	0.1061	3.9452	"	3.9452	0.5189	0.2629	3.1634	3.9452
0.400	1.2602	3.9452	"	3.9452	0.6098	0.1252	3.2102	3.9452
0.500	1.3423	3.9452	"	3.9452	0.6551	0.0558	3.2343	3.9452
0.600	1.4114	3.9452	"	3.9452	0.6702	0.0519	3.2231	3.9452
0.700	1.4021	3.9452	"	3.9452	0.6459	0.1011	3.1982	3.9452

TABLE II
Nitrogen balance

KH_2PO_4 added (gm.)	Yield of oven-dry yeast (gm.)	Nitrogen ingoing			Nitrogen outgoing			
		$(\text{NH}_4)_2\text{SO}_4$ (gm.)	Seed yeast (gm.)	Total (gm.)	Net yeast (gm.)	Spent media (gm.)	Nitrogen loss (gm.)	Total (gm.)
0.100	0.7502	0.1663	Negligible	0.1663	0.0413	0.0508	0.0242	0.1663
0.200	0.8311	0.1663	"	0.166	0.05493	0.0440	0.0174	0.1663
0.300	1.1061	0.1663	"	0.1663	0.0771	0.0280	0.0112	0.1663
0.400	1.2602	0.1663	"	0.1663	0.0931	0.0174	0.0058	0.1663
0.500	1.3423	0.1663	"	0.1663	0.1017	0.0106	0.0040	0.1663
0.600	1.4114	0.1663	"	0.1663	0.1035	0.0080	0.0048	0.1663
0.700	1.4021	0.1663	"	0.1663	0.0983	0.0109	0.0071	0.1663

Ferrous sulphate	0.0034 gm.
Zinc sulphate	100 microgram
Cupric sulphate	8 microgram

Distilled water to make 200 c.c.

The flasks were sterilized at 15 lb. pressure of steam for 20 minutes, cooled and seeded with a drop of fresh yeast suspension containing negligible amount of yeast. The flasks were incubated at 33° to 35° C. for 28 days after which yeast was filtered and washed well with distilled water under suction through Whatman No. 50 filter-paper. Filtrate was kept for the estimation of carbon and nitrogen. Yeast was dried to a constant weight in an oven at 100° to 105° C. for about 5 to 6 hours. Dry yeast was powdered and passed through 100-mesh sieve and treated for the estimation of carbon and nitrogen. Nitrogen and carbon were estimated by Kjeldahl and Wlakley and Blacks^s methods respectively.

TABLE III
C/N ratio

KH ₂ PO ₄ added (gm.)	Yield of oven-dry yeast (gm.)	Carbon in yeast %	Nitrogen in yeast %	C/N
0.100	0.7502	41.70	5.51	7.5
0.200	0.8311	45.72	6.60	6.9
0.300	1.1061	46.92	6.97	6.7
0.400	1.2602	48.40	7.39	6.5
0.500	1.3423	48.82	7.58	6.4
0.600	1.4114	47.50	7.33	6.4
0.700	1.4021	46.06	7.01	6.5

DISCUSSION

From our results we find that the yield of yeast regularly increases with the increase in the concentration of the phosphate until at 0.3% concentration, the maximum yield is obtained. The phosphate seems to increase regularly the formation of carbon dioxide and other volatile substances during the yeast growth upto a concentration of 0.25% after which loss

of carbon from the system is decreased. From the study of nitrogen balance we see that upto a concentration of 0.25% the loss of nitrogen is gradually decreased. It is interesting to observe that the same concentration, i.e., 0.25% which gives maximum carbon loss lowers the loss of nitrogen to minimum. As we increase the dose of the phosphate to 0.25% we get yeast with greater nitrogen and carbon content. Thus the phosphate seem to form a richer yeast with more protein and carbohydrate. As we vary the dose of the phosphate C/N ratio in yeast cells is slightly decreased. All these results are very akin to the results obtained in soil system by Dhar.⁹

SUMMARY

Influence of different concentrations of potassium dihydrogen phosphate on the carbon balance, nitrogen balance and C/N ratio in yeast cells has been studied in cultures of *Dhar yeast*. The increased doses of the phosphate decreases the nitrogen loss, increases the carbon loss and decreases C/N ratio in yeast cells. The addition of higher doses of phosphates lead to the production of a richer quality of yeast with increased nitrogen and carbon content.

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NITROGEN TRANSFORMATIONS IN SOIL WITH COAL

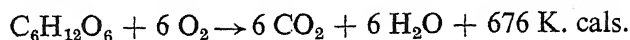
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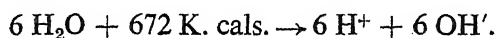
Read at the 26th Annual Session of the Academy held at the Aligarh Muslim University
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COAL is considered to be formed from wood and other plant materials which have been gradually dehydrated by the pressure of the superimposed layer of earth fallen on them. Although ordinary plant materials easily decay and undergo oxidation, the lignin extracted out of them is not so easily affected. Lignin contains much less loss of hydrogen and oxygen than cellulose and other carbohydrate constituents of plants. It therefore appears that oxidation becomes more and more difficult as the stages of dehydration proceed from the simple carbohydrate, glucose to one of the most complex one anthracite coal, which as is well known is one of the most difficult substances for oxidation at ordinary temperature. Therefore coal is an old matter containing cellulosic and lignin substances with protein. The constitution of these has changed due to the flux of time resulting in an inert product. Coal though representing tremendous accumulation of humus has not been used in agriculture due primarily to its inert nature. It has now been well established that cellulose, lignin, etc., undergo oxidation when added to the soil. Similarly coal though old matter should also be oxidised. As alkalies help oxidation, the oxidation of different varieties of coal when added to the soil will be more easy in tropical soils which have a tendency to be alkaline than in soils of temperate countries which are acidic. Although coal appears to be rather dead there is one redeeming feature in coal; that it adds large quantities of carbonaceous substances. And hence on the addition of coal to a soil, the available soil nitrogen is not likely to be converted into microbial cells, which compete with the growing crops, as happens when cellulose lignin or fat is added to the soil. Moreover coal can help better aeration and increased water retention capacity of the soil. It has been found by Gaddini¹ that the distribution of powdered lignite with 7-10% humus improves the fertility, the humus content, physico-chemical properties, structure and microbial activity of the soil. It appears therefore that coal can prove to be moderately good nitrogenous manure specially in tropical soils when either added in very fine state of division alone or mixed with other energy-rich materials.

Although various workers tried to explain the fixation of nitrogen from different angles, *e.g.*, bacterial, chemical, etc., but in countries like India where the temperature, and sunlight play an important role, much of the facts which otherwise remain unexplainable are easily explained from photochemical view of nitrogen fixation. According to this theory, a simple carbohydrate of the type glucose when oxidised gives out 676 K. cal. of heat energy per gram molecule of glucose oxidised as per following equation:



This oxidation in the soil takes place always in the presence of some moisture, which easily gets broken into H^+ and OH' thus, by the energy of oxidation:



This H^+ combines with the nitrogen of the air which is always present in the soil as follows:



The ammonia gets dissolved in the soil and gets oxidised into nitrites and nitrates in due course.

This view has been substantiated by a large number of experimental data, that cellulosic and other carbonaceous materials left in the field after harvest and added to them incidentally as manure get oxidised more in sunlight than in dark and the energy released fixed the atmospheric nitrogen in the soil as ammonia, which gradually changes into the different nitrogenous materials, as found in soils and as required by plants. Dhar and Mukherjee² have found that this fixation can take place not only in unsterile soils but also in sterilised soils on the addition of energy-rich materials. Pant,³ Kapoor,⁴ Chatterji⁵ and other coworkers of Dhar have found that energy-giving materials as cellulose, green leaf, etc., get oxidised in soil and fix nitrogen.

We have therefore tried to show in this paper that coal which is cheap in its cost and easily available to farmers can conserve nitrogen and improve the nitrogen status of the soil.

Three grams of well powdered coal per pound of the soil was taken with 50 ml. of water and was mixed with soil containing 3 gm. per pound of ammonium nitrate. Another set containing 3 gm. of coal added per pound of soil was taken. The samples were taken in duplicate in glass jars and these were exposed daily for eight hours in sunlight.

Analysis of coal (Lignite)

Total Carbon	.. 66.00%
Total Nitrogen	.. 0.7670%
Ammoniacal Nitrogen	.. 0.0260%
Nitrate Nitrogen	.. 0.0271%
Total Sulphur	.. Traces
Ash	.. 71.40%
Moisture	.. 22.9%

Analysis of Coal Ashes

Silica	.. 45.4%
Alumina	.. 30.2%
Calcium oxide	.. 5.5%

TABLE I

Exposed Set—Average Temperature 30° C.
1 pound of Soil + 3 gm. of ammonium Nitrate

Period of Exposure	Total Carbon %	Total Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	0.0348	0.0004	0.0010	..
0 Day ..	0.4043	0.2658	0.1531	0.1545	..
1 Month ..	0.3885	0.1595	0.0763	0.0898	39.99
2 Months ..	0.3736	0.1431	0.0691	0.0788	46.16
3 Months ..	0.3591	0.1266	0.0587	0.0678	52.38
4 Months ..	0.3441	0.1152	0.0520	0.0602	56.66
5 Months ..	0.3295	0.1037	0.0452	0.0527	60.99
6 Months ..	0.3137	0.0968	0.0413	0.0479	63.58
7 Months ..	0.2980	0.0899	0.0373	0.0431	66.17
8 Months ..	0.2838	0.0792	0.0338	0.0383	70.20

TABLE II

Exposed Set—Average Temperature 30° C.
1 pound of Soil + 3 gm. of Coal + 3 gm. of Ammonium Nitrate

Period of Exposure	Total Carbon %	Carbon Oxidised %	Total Nitrogen %	Decrease in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010	..
0 Day ..	0.8379	..	0.2708	..	0.1533	0.1546	..
1 Month ..	0.8061	0.0318	0.1671	0.1037	0.0834	0.0656	38.29
2 Months..	0.7743	0.0636	0.1637	0.1071	0.0810	0.0603	39.65
3 Months..	0.7432	0.0947	0.1595	0.1113	0.0756	0.0549	41.10
4 Months..	0.7105	0.1274	0.1557	0.1151	0.0672	0.0495	42.50
5 Months..	0.6787	0.1592	0.1519	0.1189	0.0587	0.0486	43.90
6 Months..	0.6452	0.1927	0.1481	0.1227	0.0502	0.0475	45.30
7 Months..	0.6125	0.2254	0.1443	0.1265	0.0438	0.0463	46.70
8 Months..	0.5824	0.2555	0.1379	0.1329	0.0369	0.0443	49.08

TABLE III

Exposed Set—Average Temperature 30° C.
1 pound of Soil + 3 gm. of Coal (Lignite)

Period of Exposure	Total Carbon %	Carbon oxidised %	Total Nitrogen %	Increase in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010
0 Day ..	0.8379	..	0.0398	..	0.0004	0.0010
1 Month ..	0.8044	0.0335	0.0405	0.0007	0.0004	0.0010
2 Months ..	0.7708	0.0671	0.0411	0.0013	0.0004	0.0010
3 Months ..	0.7371	0.1008	0.0419	0.0021	0.0004	0.0010
4 Months ..	0.7093	0.1286	0.0426	0.0028	0.0005	0.0010
5 Months ..	0.6740	0.1639	0.0433	0.0035	0.0005	0.0010
6 Months ..	0.6396	0.1983	0.0433	0.0035	0.0005	0.0010
7 Months ..	0.6033	0.2346	0.0438	0.0040	0.0005	0.0010
8 Months ..	0.5688	0.2691	0.0443	0.0045	0.0005	0.0010

DISCUSSION

That coal can be used as a conservator of nitrogen is evident (Table II) from the percentage loss of nitrogen in the case of the decomposition of ammonium nitrate. The loss went to 49.08% in eight months time whereas ammonium nitrate with soil alone showed a loss of 70.20% of nitrogen in the same time (Table I). In the case of ammonium nitrate and coal the ammoniacal and nitric nitrogen both decreased steadily. The decrease in total nitrogen was not abrupt but was steady. The oxidation of carbon was slow, but was regular.

The nitric as well as ammoniacal nitrogen did not show any increase when coal alone was used in the beginning; after three months they increased and remained practically constant at the end. The total nitrogen also increased considerably. A increase of 11.5% of the initial nitrogen was noted after eight months whilst it increased to 5.40% in three months and 9.03% in six months time. The oxidation of carbon though slow was approximately constant. In three months it oxidised to 13.0%, in six months the oxidation was 23.7%, whilst a oxidation of 32.1% was observed in eight months time. The coal taken was a pure sample of lignite. It is quite evident from the tables that the coals are slowly oxidised in this process and the nitrogen of the air is fixed. The amount of the carbon oxidised and the increase of nitrogen even in three months much exceeds the experimental error. It seems that the introduction of coal to soil makes the soil more porous and thus the oxidation is facilitated.

It is clear therefore, that coal in finely divided state, either alone or mixed with other organic substances not only add organic matter to the soil but supply their own nitrogen and also atmospheric nitrogen by fixation when exposed to sunlight and prove to be fairly good manures, specially for tropical countries. Thus coal though inert and difficult to oxidise are believed to undergo photochemical changes and fix the atmospheric nitrogen in the soil. The value of coals as a manure is not only due to the nitrogen they contain but it is their nitrogen fixing power and contents of minerals that increase their usefulness both as a manure and as a conservator.

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NITROGEN TRANSFORMATIONS IN SOIL WITH BLOOD

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Read at the 26th Annual Session of the Academy held at the Aligarh Muslim University on February 4, 1957

AGRICULTURAL operations are confined to soil only, and therefore much attention should be given to its composition, fertility and its conservation. Although soil is a complex medium and all the known elements can be expected in it and its fertility also depends on numerous factors, but essentially the main problem of today in maintaining soil fertility is to ensure the supply of essential plant food and organic matter under optimum condition. Fertility of soil depends largely on adequate supplies of nitrogen in the form of organic and inorganic fertilisers.

In 1937 Lander¹ concluded that the organic manures produce definite effects, and in case of heavy doses, it is significant even after harvesting three crops. In the same year Vishwanath reviewed that the greatest returns are generally obtained by nitrogenous manuring.

In tropical countries, particularly in India, there is one great advantage of temperature and because of enhanced oxidation of nitrogenous and carbonaceous matter, there is a great amount of available nitrogen in tropical soils than in temperate soils. Dhar² has observed that 1-2 per cent. of total nitrogen was available in temperate soils whereas 10-30 per cent. was in the tropical ones. The availability is probably due to the presence of more nitrates and ammonia in the solution ready for absorption by the plants. He found that the easily oxidisable carbonaceous matter like molasses, cowdung, hay, etc., when added to soils, improve their nitrogen status due to fixation from atmosphere, producing thereby much more available nitrogen. And thus not only the total nitrogen content of the soil was improved, but also the ammonium and nitrate content were the highest.

Mukerji and Agarwal³ in 1950 reviewed that increased fields of paddy as a result of green manuring have been reported from many provinces. The increase in the field of cane as a result of green manuring has been estimated at about 50 per cent. over unmanured ones. Green manuring of wheat is considered feasible only in tracts where sufficient autumn rains are available or irrigation facilities exist. Patel⁴ in 1937 described some

very useful effects of green manuring as compared with FYM. Similar results were met by Panigrahi⁵ in 1950. It is thus clear that the value of artificial fertilizers should be greatly enhanced if they are mixed with organic substances. With this point of view we have tried to show in this paper that blood can also act as a good organic material and can serve as a energy-rich material whether used alone or with inorganic fertilizers with soil.

Three c.c. of goat's blood was added to one pound of soil with 50 c.c. of water. Another set contained 3 gm. of ammonium nitrate along with 3 c.c. of blood and 50 c.c. of water with one pound of soil. These sets were taken in duplicate and were exposed for eight hours daily for eight months. The moisture was maintained constant throughout and average temperature was recorded.

TABLE I

Exposed Set—Average Temperature 30° C.
1 pound of Soil + 3 gm. of Ammonium Nitrate

Period of Exposure	Total Carbon %	Total Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	0.0348	0.0004	0.0010	..
0 Day ..	0.4043	0.2658	0.1531	0.1545	..
1 Month ..	0.3885	0.1595	0.0763	0.0898	39.99
2 Months ..	0.3736	0.1431	0.0691	0.0788	46.16
3 Months ..	0.3591	0.1266	0.0587	0.0678	52.38
4 Months ..	0.3441	0.1152	0.0520	0.0602	56.66
5 Months ..	0.3295	0.1037	0.0452	0.0527	60.99
6 Months ..	0.3137	0.0968	0.0413	0.0479	63.58
7 Months ..	0.2980	0.0899	0.0373	0.0431	66.17
8 Months ..	0.2838	0.0792	0.0338	0.0383	70.20

TABLE II

Exposed Set—Average Temperature 30° C.
1 pound of Soil + 3 c.c. of Blood + 3 gm. of Ammonium Nitrate

Period of Exposure %	Total Carbon %	Carbon Oxidised %	Total Nitrogen %	Decrease in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010	..
0 Day ..	0.4394	..	0.2793	..	0.0009	0.0014	..
1 Month ..	0.4086	0.0308	0.2202	0.0591	0.0029	0.0034	21.17
2 Months..	0.3884	0.0510	0.2160	0.0632	0.0034	0.0060	22.64
3 Months..	0.3598	0.0796	0.2088	0.0704	0.0034	0.0091	25.23
4 Months..	0.3419	0.0975	0.2051	0.0741	0.0048	0.0126	26.54
5 Months..	0.3277	0.1117	0.2011	0.0781	0.0048	0.0141	27.98
6 Months..	0.3181	0.1213	0.1946	0.0846	0.0042	0.0195	30.31
7 Months..	0.3084	0.1310	0.1853	0.0939	0.0040	0.0210	33.64
8 Months..	0.2961	0.1433	0.1795	0.0997	0.0038	0.0257	35.72

TABLE III

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 c.c. of Goat Blood

Period of Exposure	Total Carbon %	Carbon oxidised %	Total Nitrogen %	Decrease in Nitrogen %	Total NH ₃ -N %	Total NO ₃ -N %	% Loss
Original Soil	0.4043	..	0.0348	..	0.0004	0.0010	..
0 Day ..	0.4394	..	0.0483	..	0.0004	0.0010	..
1 Month ..	0.4253	0.0141	0.0444	0.0038	0.0010	0.0030	8.0
2 Months..	0.4104	0.0290	0.0404	0.0078	0.0014	0.0060	16.3
3 Months..	0.3950	0.0444	0.0363	0.0119	0.0014	0.0090	24.8
4 Months..	0.3805	0.0589	0.0323	0.0159	0.0024	0.0110	33.1
5 Months..	0.3682	0.0712	0.0282	0.0200	0.0029	0.0160	41.5
6 Months..	0.0554	0.0840	0.0233	0.0249	0.0025	0.0165	51.7
7 Months..	0.3431	0.0963	0.0194	0.0288	0.0023	0.0191	59.8
8 Months..	0.3348	0.1046	0.0157	0.0325	0.0019	0.0227	67.4

DISCUSSION

Table II shows the effect of blood on the decomposition of ammonium nitrate when added to the soil. A loss of 35.72 per cent. of total nitrogen was met with blood, but a loss of 70.20 per cent. of nitrogen was obtained when ammonium nitrate was mixed with the soil all alone (see Table I). This clearly indicates that goat's blood which goes always as a waste can be used safely as a powerful conservator.

The oxidation of carbon was 7.0 per cent. in the first month, 18.1 per cent. in the third month, 26.7 per cent. in the sixth month and 32.6 per cent. in the eighth month respectively. Similarly, a loss of 21.17 per cent. of nitrogen was met with in the first month, 25.23 per cent. in the third month, 30.31 per cent. in the sixth month and 35.72 per cent. in the eighth month were observed.

Table III shows the nitrogen and carbon changes when soil mixed with blood was exposed to sunlight. In three months time a loss of 24.8 per cent. of nitrogen was observed while in six and eight months time losses of 51.7 per cent. and 67.4 per cent. were met with. These results are in full agreement with the results obtained by different workers at different experimental stations. A marked increase both in ammoniacal and nitric nitrogen were observed. The carbon oxidised to an extent of 23.8 per cent. in eight months, whilst in six and three months time the oxidation was 19.1 per cent. and 10.1 per cent. respectively.

A comparison of the percentage loss of total nitrogen in the case of molasses, sawdust, wheat straw, coal and goat blood along with soil + ammonium nitrate (see Previous Publications) all in equal quantities, *e.g.*, 3 gm./pound of soil shows very clearly that the loss of nitrogen is minimum in the case of blood + maximum in the case of sawdust. The percentage loss of nitrogen is of the following order:

Blood > Molasses > Wheat straw > Coal > Sawdust.

The percentage oxidation of the carbon is however of the reverse order, *i.e.*,

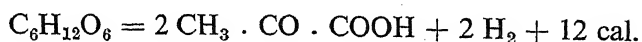
Blood < Molasses < Wheat straw < Coal < Sawdust.

That the loss of nitrogen in the case of blood is least can be explained on the basis that this nitrogenous substance, apart from containing carbonaceous matter also contains a large amount of fat, and fats are well known to retard the oxidation of nitrogenous compounds added to the soil. This decrease in the velocity of oxidation of nitrogenous substances lessens the possibility of formation and decomposition of the unstable intermediate

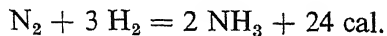
compound ammonium nitrite to nitrogen gas and thus decreases the loss of nitrogen.

The percentage oxidation of carbon in the above cases can be explained from the fact that the percentage of carbon oxidised in the lower concentrations is more than the higher concentrations. The reason seems to be that for equal amount of surface in higher and lower concentrations of energy materials, the molecules of the energy materials in the higher concentrations are more compact per unit area of the surface leaving proportionately smaller spaces for adsorption of the gases of the atmosphere. Hence for every molecule of the energy material there are proportionately a lesser number of molecules of oxygen available for the oxidation of carbon in the higher concentrations than in the lower ones.

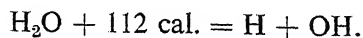
Similarly, a comparative study of total ammoniacal and total nitrate nitrogen can be made in the above cases. (See "Nitrogen Transformations in Soil by Molasses, Sawdust, Wheat Straw, Coal and Blood" by the above authors in their previous publications.) In every case a decrease in nitrogen status both in ammonical and nitric form is continuous. These losses are again heaviest in the case of sawdust and least in the case of blood. This clearly indicates why the total loss of nitrogen in the case of blood is least and it decreases from blood to molasses, molasses to wheat straw, wheat straw to coal and from coal to sawdust. The reason is very clear and depends on the oxidation of carbohydrates. If a simple carbohydrate, say glucose, is taken and allowed to oxidise, it readily oxidises to form pyruvic acid and gives hydrogen under an aerobic condition according to the following equation:



In the presence of nitrogen of the atmosphere and on the soil surface, the hydrogen obtained from decomposition of glucose can form ammonia according to the equation:



The presence of oxygen, as under ordinary conditions, it is difficult for the production of hydrogen from glucose. Hence in order to obtain atomic hydrogen required for ammonia formation, the following reaction has to take place:



In other words, for obtaining a molecule of NH_3 according to the above equation, 336 calories are needed. In photosynthesis also, the breaking up

of water molecule into $H + OH$ may be involved in nitrogen fixation may be the same breaking up of a water molecule. The energy required for this breaking up of water is derived from the oxidation of energy materials needed for nitrogen fixation. Hence in the process of nitrification in the above substances, which is accelerated by light, there is more loss of nitrogen in the gaseous state, than the amounts of nitrates formed, and nitrification and loss of nitrogen are markedly seen in the above ratio.

It is therefore quite clear from the above results that the value of artificial fertilizer should be greatly enhanced if they are mixed with energy-rich materials like molasses, sawdust, wheat straw, coal and blood, which although very cheap and easily available to farmers in general are often overlooked. The greater value of organic nitrogenous compounds or a mixture of ammonium salts and carbonaceous substances for the soil than ammonium salts alone lies in the fact that not only soil texture is improved by the colloids added with organic manure, but the carbonaceous matter added acts as an agent in the preservation of nitrogen compounds of the soil by behaving as a negative catalyst.

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SEARCH FOR NEW AMOEBICIDES

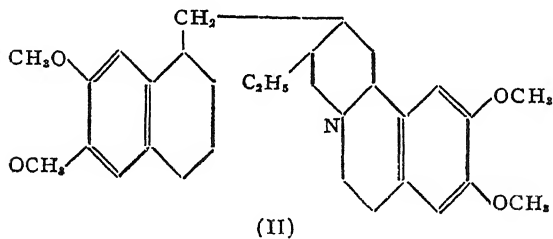
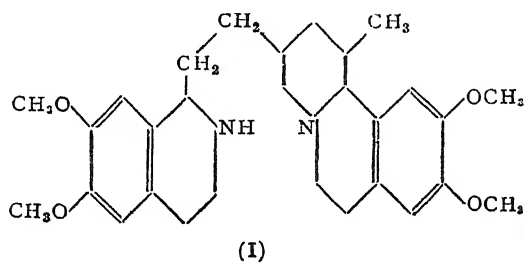
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EMETINE possesses high amoebicidal activity but is highly toxic and a depressant. Moreover, it cannot kill the encysted form of *E. histolytica* and is of little use in chronic dysentery. This necessitates the search for a better drug than emetine.

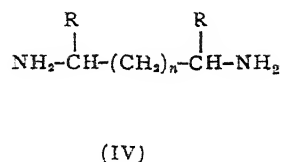
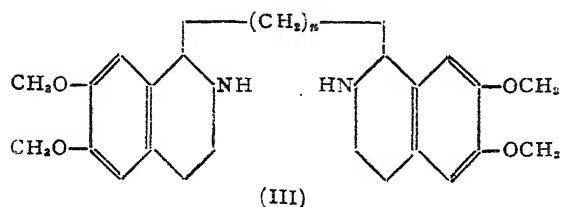
The structure of emetine was advanced by Brindley and Pyman¹ as (I) but Robinson,² Pailer,³ Battersby, Openshaw and Wood⁴ corrected it to (II).



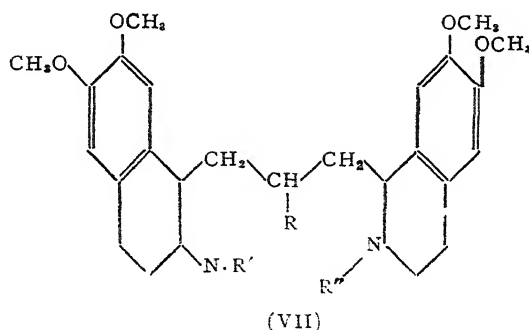
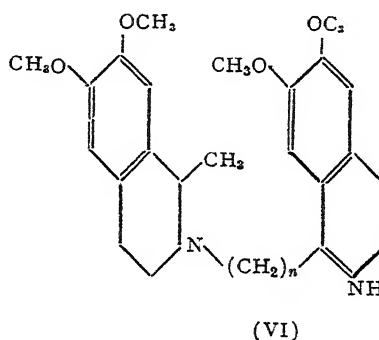
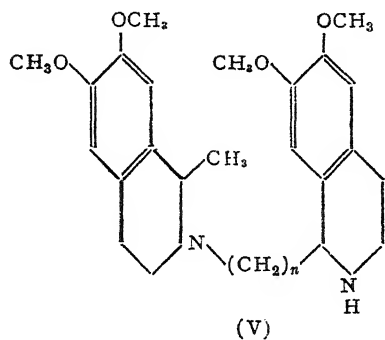
Based on the structure of Brindley and Pyman⁵ Child and Pyman⁶ synthesised a number of amines of the type (III, $n = 4, 5$ and 8) which could be considered to be derived from emetine molecule. These compounds did not show any appreciable amoebicidal activity.

Pyman⁷ also synthesized and tested a large number of diamines. Some of these compounds were appreciably active *in vitro*. Goodwin *et al.*⁸ observed that among these compounds α -*k*-tetra-*n*-amyl-di-aminodecane dihydrochloride, when tested *in vitro* showed 3-5 times activity as that of emetine, though its *in vivo* activity was feeble. The work was extended by Hall *et al.*⁹ who observed remarkable *in vitro* activity in some di-amines of

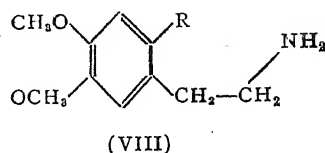
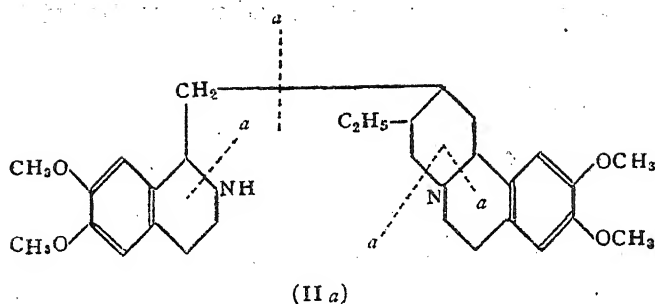
he type (IV) where $n = 3$, $R = n$ -amyl, n -hexyl or 2-ethyl hexyl; $n = 4$, $n = n$ -hexyl).



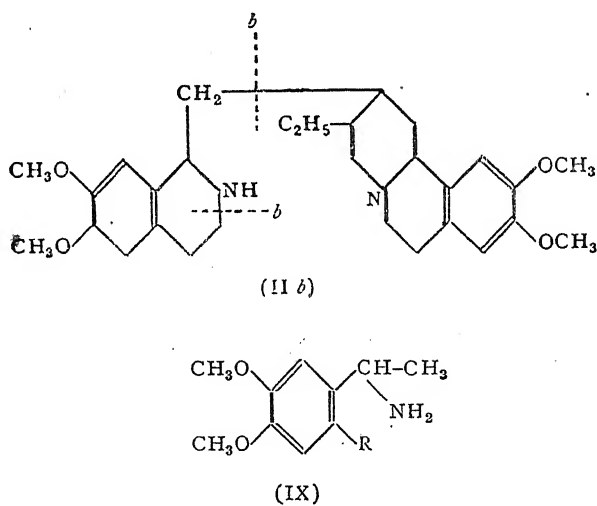
Osbond¹⁰ synthesized several substituted isoquinolines with a view to examine their amœbicidal activity. Among them compound (V, $n = 10$) was active at 1 in 10^6 , compounds (VI, $n = 4, 5$ and 10) were active at 1 in 10^3 to 10^4 , compounds (VII, $R = C_3H_7$, $R' = R'' = H$; $R = C_2H_5$, $R' = R'' = CH_3$) were active at 1 in 10^4 whereas under identical conditions emetine hydrochloride was active at 1 in $10^6 - 5 \times 10^6$.



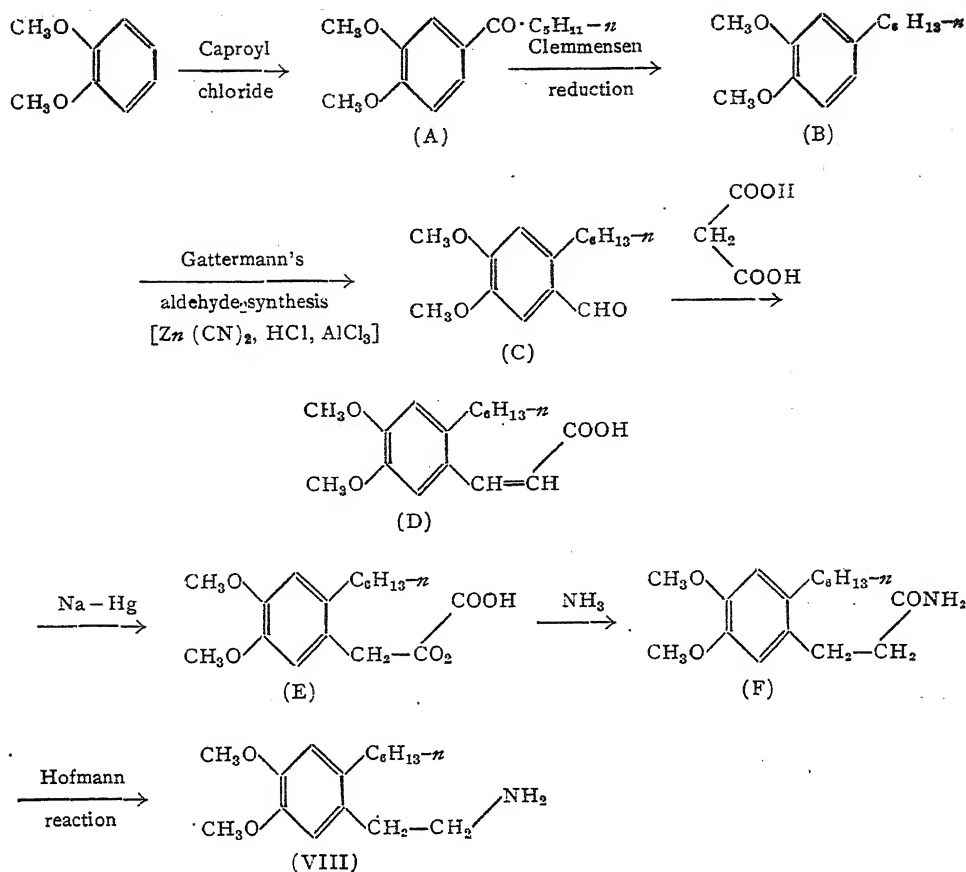
If one considers the hypothetical opening in the emetine molecule (II a) along the dotted lines (a), it would generate a compound of the type (VIII, $R = \text{alkyl group}$).



Again considering the opening in the emetine molecule (II *b*) along the dotted lines (*b*), a compound of the type (IX, R = alkyl group) may be considered to have been formed from a part of the emetine molecule.



Synthesis of compounds (VIII and IX, R = C₆H_{13-n}) have been described in this paper. β -(2-*n*-hexyl-4:5-dimethoxy phenyl)-ethyl amine (VIII, R = C₆H_{13-n}) has been prepared according to the following scheme.



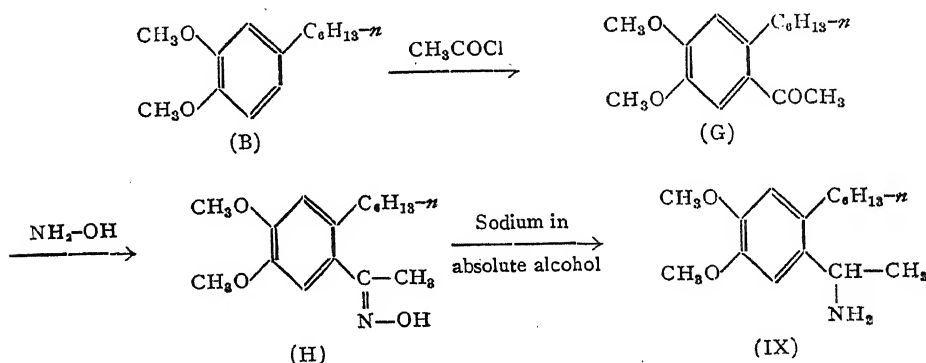
Acetoveratrone was prepared by Pictet and Gams¹¹ by condensing veratrole with acetyl chloride in presence of anhydrous aluminium chloride in good yield. Application of the same method for the preparation of 3:4-dimethoxy-phenyl-*n*-amyl ketone (A) by the condensation of veratrole with *n*-caproyl chloride in presence of anhydrous aluminium chloride yielded only 15% of the desired ketone and a good amount of tarry mass. However, it has been found that if the above condensation be conducted in presence of anhydrous zinc chloride at the boiling temperature of carbon disulphide, the yield of the ketone amounts to 49% of the theory and a negligible amount of tarry matter is formed.

Clemmensen reduction of the above ketone (A) has been effected in good yield by following a method suggested by Berger and Silberschmidt.¹² On subjecting 1-*n*-hexyl-3:4 dimethoxy-benzene (B) to Gattermann's aldehyde synthesis as modified by Adams,¹³ a yield of 30% of 2-*n*-hexyl-4:5

dimethoxy-benzaldehyde (C) has been obtained. This aldehyde on allowing to react with malonic acid in presence of piperidine (Knoevenagel modification of Perkin's¹⁴ reaction) yielded 2-*n*-hexyl-4:5-dimethoxy-cinnamic acid (D).

The above cinnamic acid has been reduced to β -(2-*n*-hexyl-4:5-dimethoxy phenyl)-propionic acid (E) by sodium amalgam in 94% yield. Conversion of the acid (E), to the corresponding amide (F), has been effected by passing a stream of dry ammonia gas through dry ammonium salt of the acid at 210° for two hours. This method was used by Haworth and Perkin¹⁵ in the preparation of β -3:4-dimethoxy-phenyl-propionamide from the corresponding propionic acid. The last stage of the synthesis, namely Hofmann reaction with amide (F), has been very unsatisfactory. Normal method of conversion, *i.e.*, treatment of the amide with aqueous hypochlorite or hypobromite yields only traces of the amine (VIII). However, conducting Hofmann reaction with bromine in sodium methoxide in methanol according to Jeffrey's¹⁶ method resulted in a better yield (30%) of the amine.

The other amine (IX, R = C₆H₁₃-*n*) has been prepared according to the scheme shown below:



1-*n*-Hexyl-3:4-dimethoxybenzene (B) on condensing with acetyl chloride in presence of anhydrous aluminium chloride yielded a ketone mainly in the form of a demethylated product. On remethylation the total yield of 2-*n*-hexyl-4:5-dimethoxyacetophenone (G) was 25% of the theoretical value. This ketone has been converted to the corresponding oxime (H) in the usual manner. The crude oxime on reduction with metallic sodium in superdry alcohol generates α -(2-*n*-hexyl-4:5-dimethoxy phenyl)-ethylamine (IX) in 58% yield.

The amoebicidal activity of the amines is under investigation and will be communicated later.

EXPERIMENTAL

Melting point's are uncorrected.

3:4-Dimethoxy-Phenyl-*n*-amyl-ketone (A): Method I

Veratrole (15 g.), *n*-caproyl chloride (15 g.), carbon disulphide (100 c.c.) and anhydrous zinc chloride (15 g.) were refluxed on a water-bath for six hours and left overnight. Carbon disulphide was then removed by distillation and the residue was decomposed by water. It was boiled for an hour, cooled and the products extracted with benzene. The benzene solution was washed with water, dried over fused calcium chloride, the solvent was removed and the residual oil was distilled under vacuum.

Boiling point 150°/1 mm.: yield was 12 g. (49%). The ketone could not be induced to crystallise.

Its *semicarbazone* on crystallisation from alcohol melted at 178°, (Found: N, 14.4; $C_{15}H_{23}O_3N_3$ requires N, 14.4%.)

Method II

Veratrole (15 g.) and *n*-caproyl chloride (15 g.) were dissolved in carbon disulphide (50 c.c.) and anhydrous aluminium chloride (10 g.) was then added portionwise, the flask being kept in ice-cold water. It was then kept for seven hours at 0° with occasional shaking and later on it was warmed for thirty minutes. The contents of the flask were poured on crushed ice to decompose the complex. Carbon disulphide layer was separated and the aqueous layer was extracted with a further amount of carbon disulphide. From the combined carbon disulphide solution, the solvent was removed and the residue was dissolved in benzene. The benzene layer was washed with sodium hydroxide solution, dried over anhydrous calcium chloride, the solvent was removed and the residual oil on distillation yielded 3.6 g. of the ketone (yield 15%).

1-*n*-Hexyl-3:4-dimethoxy-benzenze (B)

Granulated zinc (200 g.) was amalgamated in the usual manner with 5% aqueous mercuric chloride solution (400 c.c.). Amalgamated zinc thus prepared was covered with 10% hydrochloric acid and was brought to a boil. 3:4-Dimethoxy-phenyl-*n*-amyl ketone (A, 70 g.) was then added portionwise during the course of an hour. Refluxing was continued for six hours, during which period concentrated hydrochloric acid (330 c.c.) was added at regular intervals and finally refluxed for further five hours. After cooling,

the product was extracted with ether, the ether layer was dried over anhydrous calcium chloride and the solvent was removed. The residual oil on distillation under vacuum gave (B, 51 g.)

Boiling point $134-36^{\circ}/2$ mm. yield, is 77%.

(Found: C, 75.81; H, 10.18. $C_{14}H_{22}O_2$ requires C, 75.67; H, 9.91%.)

2-n-Hexyl-4:5-dimethoxy-benzaldehyde (C)

Dry hydrogen chloride was bubbled through a mixture of 1-*n*-hexyl-3:4-dimethoxy benzene (B, 33 g.), zinc cyanide (35 g.) and anhydrous benzene (180 c.c.) at 0° for one hour. Powdered anhydrous aluminium chloride (22 g.) was added to the mixture and hydrogen chloride was passed for further three hours at 0° and then for half an hour at $40-50^{\circ}$ and finally for two hours at $50-60^{\circ}$. Next day, the mass was decomposed by ice and refluxed for fifteen minutes. Benzene layer was then separated and the aqueous layer was again refluxed for two hours. It was cooled and extracted with benzene. The combined benzene layers were dried over anhydrous calcium chloride, benzene was removed by distillation and the residual oil was distilled under vacuum. The aldehyde distilled at $168-70^{\circ}/2$ mm.; yield was 11 g. (30%). 2:4-dinitro-phenyl-hydrazone crystallised from alcohol, m.p. 210° . Its *semicarbazone* crystallised from alcohol and melted at $134-35^{\circ}$.

(Found: N, 13.8. $C_{16}H_{25}O_3N_3$ requires N, 13.5%.)

2-n-Hexyl-4:5-dimethoxy-cinnamic acid (D)

2-*n*-Hexyl-4:5-dimethoxy-benzaldehyde (20 g.), malonic acid (18 g.), pyridine (40 c.c.) and piperidine (1 c.c.) were refluxed on a water-bath for three hours and then on an asbestos-centred wire-gauze for one hour. It was cooled and poured into a mixture of crushed ice and concentrated hydrochloric acid (60 c.c.). The desired cinnamic acid precipitated out. It was crystallised from dilute alcohol. Melting point $95-96^{\circ}$; yield 18 g. (77%).

(Found: C, 70.1; H, 8.3; OCH_3 , 21.42. $C_{17}H_{24}O_4$ requires C, 69.86; H, 8.21; OCH_3 , 21.23%.)

β -(2-n-Hexyl-4:5-dimethoxy-phenyl)-propionic acid (E)

2-*n*-Hexyl-4:5-dimethoxy-cinnamic acid (17 g.) was dissolved in dilute sodium hydroxide solution. It was reduced with excess of sodium amalgam (3.5%) in the usual manner. After the reaction was over, the solution was acidified with concentrated hydrochloric acid when an oil separated out. It soon solidified and on crystallisation from dilute alcohol melted at $70-72^{\circ}$. Yield, 16 g. (94%).

(Found: C, 69.1; H, 9.1. $C_{17}H_{26}O_4$ requires C, 69.4; H, 8.9%.)

β-(2-*n*-Hexyl-4:5-dimethoxy phenyl)-propionamide (F)

Dry ammonia gas was passed through dry ammonium salt of *β*-(2-*n*-hexyl-4:5-dimethoxy phenyl)-propionic acid (E) at 210° for a period of two hours. It was then poured in cold water, when a solid separated out. The amide on crystallisation from dilute alcohol melted at 58–60°. Yield, 60% of the theory.

(Found: N, 4.6. $C_{17}H_{27}O_3N$ requires N, 4.7%.)

β-(2-*n*-Hexyl-4:5-dimethoxy phenyl)-ethylamine (VIII)

β-(2-*n*-Hexyl-4:5-dimethoxy phenyl)-propionamide (5 g.) was dissolved in dry methanol (10 c.c.). This was added to a solution of sodium methoxide prepared out of sodium (0.8 g.) and methanol (30 c.c.). To the cold solution, bromine (2.6 g.) was added and the mixture was heated on a water-bath for half an hour. The solution was made just acidic with the addition of acetic acid and methanol was removed by distillation. The residual mass was then hydrolysed with caustic soda solution (25%). It was then extracted with benzene and the benzene solution was twice extracted with dilute hydrochloric acid. The combined acid solution was made alkaline with sodium hydroxide solution and the separated oil was extracted with ether. The ether solution was dried over solid caustic potash; on addition of saturated solution of dry hydrogen chloride in ether to the ethereal solution of the amine, hydrochloride of the amine separated out. On crystallisation from absolute alcohol, it melted at 123–24°; yield, 1.5 g. (30%).

(Found: C, 63.8; H, 9.4; N, 4.7. $C_{16}H_{26}O_2NCl$ requires C, 63.7; H, 9.3; N, 4.6%.)

Picrate on crystallisation from dilute alcohol melted at 162–63°.

2-*n*-Hexyl-4:5-dimethoxy-acetophenone (G)

1-*n*-Hexyl-3:4-dimethoxy benzene (B, 20 g.), acetyl chloride (10 g.) and carbon disulphide (100 c.c.) were cooled to 0°. Anhydrous aluminium chloride (30 g.) was added to this well-cooled mixture in small portions at a time. It was kept for six hours in ice-bath with occasional shaking. The contents of the flask were then warmed for few minutes. Carbon disulphide was distilled off and the residue was decomposed by ice. It was then extracted with benzene. The residue on removal of benzene was found to consist mainly of the demethylated product. The whole product was therefore methylated with methyl sulphate in the usual manner. The methylated product was extracted with benzene and the benzene solution was

dehydrated over anhydrous calcium chloride. Removal of the solvent and subsequent distillation under vacuum yielded the ketone (6 g.; 25%). Boiling Point $162-65^{\circ}/2$ mm.

(Found: C, 72.1; H, 9.4 $C_{16}H_{24}O_3$ requires C, 72.7; H, 9.1%.)

Its *semicarbazone* could not be isolated as a solid.

*α -(2-*n*-Hexyl-4:5-dimethoxy phenyl)-ethyl amine (IX)*

2-*n*-Hexyl-4:5-dimethoxy-acetophenone (G, 5 g.), hydroxylamine hydrochloride (2.5 g.) and sodium hydroxide (1.5 g. in 10 c.c. water) were dissolved in minimum amount of alcohol by heating on a water-bath under reflux. After refluxing for four hours, it was diluted with water and the oxime was extracted with ether. The ethereal solution was dehydrated over anhydrous sodium sulphate and on removal of the solvent, a viscous oil (4 g.) was left. The oil did not solidify and it was reduced for the preparation of the amine (IX).

Crude 2-*n*-hexyl-acetophenone oxime (4 g.) was dissolved in super-dry alcohol (100 c.c.). To the hot solution metallic sodium (4 g.) was added rapidly to maintain a vigorous reaction. After the whole of sodium had dissolved, the reaction mixture was cooled. The solution was made acidic with concentrated hydrochloric acid and alcohol was removed by distillation under reduced pressure. The residual mass was dissolved in water, basified with sodium hydroxide solution and the amine was extracted with benzene. Benzene layer was washed with water and later on adding concentrated hydrochloric acid to the benzene layer, the hydrochloride of the amine separated out. The solid was filtered, washed with benzene and crystallised from hot water. It melted at 178° . Yield, 2.5 g. (58%).

(Found: C, 63.6; H, 9.5; N, 4.3. $C_{16}H_{28}O_2NCl$ requires C, 63.7; H, 9.3; N, 4.6%.)

SUMMARY

β -(2-*n*-hexyl-4:5-dimethoxy-phenyl)-ethylamine and α -(2-*n*-hexyl-4:5-dimethoxy-phenyl)-ethylamine have been prepared with an object to observe their amoebicidal activity. The first amine has been prepared by the following route: veratrole \rightarrow 3:4-dimethoxy-phenyl-*n*-amyl ketone \rightarrow 1-*n*-hexyl-3:4-dimethoxy-benzene \rightarrow 2-*n*-hexyl-4:5-dimethoxy-benzaldehyde \rightarrow 2-*n*-hexyl-4:5-dimethoxy-cinnamic acid \rightarrow β -(2-*n*-hexyl-4:5-dimethoxy-phenyl)-propionic acid \rightarrow amide \rightarrow β -(2-*n*-hexyl-4:5-dimethoxy-phenyl)-ethyl amine. The second amine has been synthesized as follows: 1-*n*-hexyl-3:4-dimethoxy-benzene \rightarrow 2-*n*-hexyl-4:5-dimethoxy-acetophenone \rightarrow oxime \rightarrow amine.

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